

Two wishes for the zero option

Mr George Bush has spoken up (but not well) in Europe, and has won public agreement that no intermediate-range nuclear missiles would be best. But that is wishful thinking.

Mr George Bush, the Vice-President of the United States, seems to have done well in persuading his European hosts these past two weeks that the best strategy for controlling intermediate-range nuclear weapons in Europe is to do away with them altogether. Chancellor Helmut Kohl agreed with that proposition last week in Berlin and then, in London at the weekend, agreed with Mrs Margaret Thatcher, the British Prime Minister, that the zero option is indeed the best possible option. The trouble is that it may not be feasible, which is no doubt why everybody has taken to saying that flexibility is also important.

In the past few weeks, in an almost unprecedented exercise in diplomatic negotiation by means of public speeches, the Soviet Union has made clear that the zero option in its strictest sense is unacceptable. The Soviet position is clear. If the planned deployment in Western Europe of Pershing II and cruise missiles were abandoned, the Soviet force of SS20 missiles would be reduced to the numerical equivalent of the British and French nuclear forces. The strict zero option, first defined last year by President Reagan, would do away with all SS20 missiles without reference to the British and French nuclear forces. As in other negotiations of this kind, each side is by its own lights on firm ground. How will it ever be possible to reach agreement?

The first need is to acknowledge what is the most hopeful sign — the willingness of both sides to consider very substantial reductions of nuclear forces. In all previous agreements of this kind, springing from the strategic arms limitation talks of the 1970s, the objective has been merely to negotiate a ceiling for the numbers of intercontinental missiles which is comfortably above anything likely to be achieved in the foreseeable future. The United States, on behalf of the North Atlantic Treaty Organization (NATO), is now pressing to be prevented from deploying intermediate-range weapons of any kind in Western Europe. The Soviet Union, preoccupied by what it considers to be the threat of the British and French nuclear forces, is nevertheless prepared to get rid of two-thirds of its nuclear weapons of intermediate range. That, surely, is progress of a kind.

The second need is that each side should try harder to understand the other's position. (Making public speeches on the subject, by making flexibility less accessible, is a bad habit that should be avoided at this stage.) The West's position is the easier to understand. The rapid deployment of the SS20 missile force during the late 1970s has substantially changed the balance of military strength in Europe, creating the spectre of circumstances in which the threat of open attack might cause the United States to reconsider its commitments under the North Atlantic Treaty. To its credit, the United States, in 1978 and 1979, fell in with the sense of fright that the SS20 missile force engendered, and agreed to deploy a force of missiles of intermediate range of its own manufacture. The plan was made public at the end of 1979, but made conditional on failure to achieve an East-West agreement. Surely, the argument goes, if the Soviet Union had felt as strongly, all these years, about the threat of Pershing II and cruise missiles, it might have said so sooner.

The Soviet position is also plausible. The SS20 missiles probably owe their existence to distant events such as the Cuban missile crisis of 1962, and some determination then in Moscow that the Soviet Union should never again find itself at the uncomfortable end of a military balance of any kind. In these

terms, the Soviet perception of Western Europe is probably of a subversive bridgehead (toehold?) on the western seaboard of Eurasia, containable by other than strategic means. And while everybody knows that in a nuclear war there are no victors, in the preliminaries to such a war, potential losers may declare themselves as such. The SS20 force is thus good strategic sense. To abandon it for good, and by binding treaty, would entail sacrifice, especially while the British and French nuclear forces remain in being. That is what the zero-option brigade must understand.

The trick at Geneva in the next few weeks must therefore be to find a compromise between these radically different opinions. The simple solution is that which has been apparent for the past few months — that the negotiators at Geneva and their governments should recognize that, so far as Western Europe is concerned, the INF ("intermediate nuclear forces") negotiations are about strategic matters. Put bluntly, the only circumstances in which the British and French governments would sanction the use of their nuclear forces are those in which Western Europe is threatened with destruction or occupation and in which the United States is judged to be irrelevant. This forecast is matched by the probable performance of the delivery systems concerned, none of which is accurate enough to carry through a counter-force strategy as distinct from deterrence. The obvious course for the people at Geneva to follow is therefore to agree that it no longer makes sense to talk about INF and strategic nuclear forces separately, but to roll both sets of negotiations together. For what it is worth, so much has been obvious from the beginning.

Diplomatically, there are two immediate problems. First, can the governments of Britain and France be persuaded that while their nuclear forces may not be obviously part of the INF negotiations, there must be some level of negotiation at which they must be counted? And, second, can enough progress be made within the year for the planned deployment of the NATO force to be avoided? The simple answers are respectively yes (by making Britain and France non-voting participants) and no (there is not enough time). The more complicated answer, for the West, is to insist that there is no logical basis on which the two sets of talks at Geneva can be separated, to acknowledge that while the zero option may be the most desirable of all possible options, it is not necessarily the best, and to plead continuing hardware failures (real enough) as reasons why the decision should be postponed by, say, six months. What, after all, is that compared with every-body's lifetime?

How to keep monopoly

British Telecom, soon to be private, could be a more powerful monopoly.

LIKE some mythical hero, Britain's Department of Industry has tried three times to tame the giant. But British Telecom (BT), even more than its fellow giant in the United States, looks like becoming more powerful the more it is hacked up. The possibility exists that any organization which has its grip on a nation's telecommunications system cannot be conquered. It can be made to tolerate competitors but not ever to be equal to them.

When the Conservatives came to power in 1979, they broke the then British Post Office into posts and telecommunications



halves, renamed the second British Telecom and broke its monopoly. The bill allowing competition in the provision of telecommunications networks and equipment was said by Mr Kenneth Baker, the industry minister, to be the most important in the life of this government.

A year later it was time to announce a second most important new bill. British Telecom was to be "privatized" — that is, 51 per cent of its shares were to be sold off to private investors and BT was to be a private corporation, existing under licence from a new Office of Telecommunications (something like the American Federal Communication Commission) just like any number of other companies the government might choose to license. But the bill is making very slow progress in the committee stage in the House of Commons, and for good reason. What it does is to turn BT from a public monopoly into a private one. The new office, called Oftel, will not have power to veto its rate rises, nor to oblige it to carry unprofitable services.

BT is unlikely ever to command less than 80 per cent of the telecommunications market. Already it has leapt into many of the fields in which it can have rivals while holding on to what monopoly it can, as in the provision of switchboards under 100 lines. What was to be the most severe blow, a competing trunk telecommunications network, is a mockery. The Department of Industry has picked one competitor — Mercury, a consortium of banks and Cable and Wireless. Mercury is dependent on BT for interconnection, domestic or international. And the protracted negotiations over the rates Mercury would have to pay BT gave BT a chance to improve its services to the City of London, one of Mercury's prime business targets.

Will any of these work? The breaking-up of AT&T in the United States so far has left 95 per cent of the telecommunications business in the AT&T's hands. The creation of the new American Bell by lopping off the comparatively unprofitable regional operating companies could moreover stimulate this AT&T fragment to clobber competitors on information services. And what the British government proposes could leave BT, on its smaller scale, much more powerful than AT&T. The new Oftel is to have nothing like the power of FCC or the state regulatory commissions to limit BT's rate of return on investment or to refuse to let it deliberately underwrite its competitors. And the new director of Oftel is to have no real power of his own (and no salary of industrial scale: £30,000 a year is being talked about). He will be able to license BT — with the approval of the Secretary of State for Industry. That makes him liable to total shackling by a Secretary of Industry in any change of government.

To this giant, rival satellites and competing trunk line rates will be just pinpricks. BT will still be a law unto itself and will be able to get away with murder — which is to say bad service, outrageous prices like the quarterly rentals of telex and power to remind anybody thinking of signing with a rival that there is no such thing as total independence from BT. AT&T might well envy BT's fate.

The government this week made big concessions to widespread fears that it is letting BT escape unregulated. It has accepted a number of recommendations in the report of Professor Stephen Littlechild of the University of Birmingham. The principal decisions are (1) that BT will be obliged, for five years at least, to keep any increase in domestic telephone rates below the increase in the retail price index and (2) that BT will need more competition to guard the public against abuse of its power.

Such competition will be stimulated in several ways. The most unexpected (and representing the biggest loss of sovereignty by BT and its related unions) is the decision to end BT's monopoly on telephone apparatus at the BT socket in the skirting board in any home. Customers will be free to buy any or all of their telephones and attachments from non-BT suppliers. Until now, the government had given BT a monopoly on "the first telephone" at any premises. The government further accepts the abolition of BT's monopoly on call-routing apparatus.

The government accepts in principle two other Littlechild recommendations but wants more time to think about them. These would reduce restrictions on reselling private telecommunications circuits leased from BT and ease restrictions on

Mercury's supply of international services.

All in all, there is a curious reluctance to subject BT to regulation by statute. For example, the government will insist that BT provide interconnection with Mercury and to cellular radio networks at "appropriate access charges" but BT is left to decide what is appropriate. The only avenue of redress will be to complain to the Monopolies and Mergers Commission.

Nonetheless, Mr Kenneth Baker, Secretary of State for Industry, foresees that BT's share of the telecommunications market could be reduced to 90 per cent by 1990 and that its hold on the equipment market will drop faster, sooner.

The way the money goes

The British government's spending budget for next year says less than it could — and should.

Big spenders spend with alarming freedom. That is the first lesson to be learned from the British government's statement last week (see also page 456) of how it plans next year to spend close on £120,000 million (with another £14,700 million on servicing its debt). For this laconic claim by central government on 43.5 per cent of the wealth likely to be generated next year within the United Kingdom is reticent almost to the point of evasiveness on the details of how the money will be used. The fact that the defence budget, 13 per cent of total spending, has not yet appeared is merely the most conspicuous oversight. In what taxpayers have a right to expect will be a rational defence of spending plans, the government chooses to lump the substantial sums of money spent by some of its departments on research and development under the general heading of "central and miscellaneous". Thus the government's supposedly dedicated expenditure on environmental research is thrown together with the cost of issuing dog licences, one of the many burdens shouldered by the Department of Environment. The whole affair is a curious contrast with the publication last week of the Reagan Administration's budget for the financial year beginning next October, which contains almost unreasonably too much information, not unreasonably too little.

The difference is significant. The US budget is not a plan for government spending in the year ahead but rather a statement of what the Administration would like to spend. In the end, Congress will decide — but, on recent form, will not decide before the next financial year has begun. In Britain, on the other hand, there is no mechanism by which Parliament can seriously quarrel with what the government plans to spend. In due course, to be sure, there will be a debate in the House of Commons about the more detailed estimates which will provide an opportunity for people to make points for (or more probably against) various items in the spending plan. Defence spending, which is to increase by more than the promised three per cent in real terms, will be even more a target this year than ordinarily. But the only real argument will come when the Chancellor of the Exchequer stands up on 15 March and explains how taxpayers will be required to contribute towards public expenditure in the year ahead. And even then, although the government will be roundly condemned by its opponents for all kinds of misdemeanours (of which the chief will be its winning of the last general election), the wisdom of its plans for spending two-fifths of the nation's wealth will not seriously be challenged.

This is hardly the way in which to run a small business, let alone a large business like a government. Since last September, the British civil service has been hard at work on the details of British public spending which, when aggregated, yield the figures published last week. Some of the work is statistical in character, as for example in estimating the amount of unemployment pay that will be required in the year ahead. (The government's guess is probably better than other people's, but is still a guess.) Other parts of the spending budget, the cost of research and development for example, are, however, the consequences of deliberate decisions and could just as easily be published now as later, and not confused with the costs of collecting licence fees for dogs.

British biotechnology

UK government instructs parliament not to interfere

ALL is well with British biotechnology, and with the British government as well — that seems to be the principal message of the government's response to the critical report of the House of Commons Select Committee on Education, Science and the Arts, published last year. The committee complained that although British universities had made important contributions to the foundations of the industry, they were now so starved of funds that innovation had become encumbered and potential innovators persuaded to seek their fortunes elsewhere.

The government's response, published last week as a memorandum attached to a letter to Mr Christopher Price, MP, the committee's chairman, breaks new ground in the executive's dismissiveness of the legislature. The government says flatly that, contrary to the committee's opinion, the arrangements that it has made for supporting biotechnology within the public sector are "broadly right in scale and nature".

Acknowledging the committee's concern that the dual support system (two-way support of university research through the University Grants Committee and through the research councils) may be in danger of breaking down, the British government says that it is too soon to know how universities will respond to a recent plea that they should pay more attention to research, and that, in any case, the Secretary of State for Education and Science (Sir Keith Joseph) "shares this concern". The memorandum says that "a proportion" of the new posts to be created in British universities with uncovenanted funds will "be in disciplines which underpin biotechnology".

One of the select committee's chief recommendations was that the government should decide which government department was in charge of biotechnology. The answer, now provided, is that the Department of Industry will in future be responsible. To help the department decide how best to carry out its new responsibilities, "an Interdepartmental Committee on Biotechnology (ICBT)" has been set up, with representation from other departments, research councils, the British Technology Group and others.

After listing the coordinating committees in biotechnology now extant in both the public and private sectors, the memorandum opines that "the existing committees adequately meet the various functions for which they were set up" but goes on to say that the "present spread of

committees is not considered to be excessive".

Enamoured of committees, subcommittees and the device of a "combination of subcommittees", the government rejects the advice from the House of Commons that there should be a separate agency of government with responsibility for biotechnology. Part of the government's difficulty seems to have been that its own agencies, the University Grants Committee included, responded to the request for a response to the House of Commons by listing the committees they had already, out of diligence, set up. The gibe that there are more British nationals deliberating on biotechnology than British biotechnologists may still be valid.

The British government's response to the House of Commons report goes on to list the steps that have been taken in recent years in the exploitation of biotechnology. Its memorandum says that the Agricultural Research Council, "recognizing the great potential for agriculture if it were possible to manipulate the genes of crop plants", has set up a programme involving 40 scientists at four of its laboratories and a further fifteen at "several universities" which is "in effect a national programme". To achieve coordination, the council also "convenes an annual discussion meeting".

Migration of British biotechnologists

THE loss of British biotechnologists to posts overseas is being tackled in a study commissioned by the Science and Engineering Research Council from the Institute of Manpower Studies. The objective is to estimate the future supply and demand of people with "key skills" and to recommend whether there should be a register to keep in touch with British biotechnologists working overseas.

Richard Pearson, director of the study, stresses that it is too early for any clear impression of the importance of the trend to have emerged. But the pattern of the emigration appears to be different from that in other fields such as micro-electronics. Jobs in biotechnology are fewer and the skills required are more specific, so it is the top people (who are often "head-hunted") who leave to take them. Higher living standards and better research opportunities in the United States and elsewhere are usually sufficient reasons to persuade people to leave.

The 1981 cuts of university budgets kept down the number of research openings in the United Kingdom and probably,

Taken together with other research in food science, veterinary medicine and "a programme of commercial significance on the conversion of animal waste into protein in the form of worms", the government reckons that it is spending about £2.4 million a year on agricultural biotechnology and employing 110 of its own scientists and technicians as well as "a further 25 in universities".

Among the research councils with an interest in biotechnology, the Medical Research Council emerges predominant. Something like £17 million of the council's spending in 1980-81 is reckoned to have been relevant, including £1.7 million for the development a monoclonal antibody for the purification of interferon. The government says that the research council's concern is "to try to hold a balance between maintaining a continued strength in basic research and exploiting opportunistically those discoveries with potential health care applications".

The Natural Environment Research Council, by contrast, is described in the memorandum as "relatively minor so far as biotechnology is concerned".

On one of the most contentious arguments of the House of Commons committee — that the arrangement between the Medical Research Council and the company Celltech Ltd should be looked into — the government has nothing to say except that the matter is being attended to. It emerges that the agreement is due to be reviewed during 1983, "two years before it expires", and that the exclusivity of the deal is on the agenda. Moreover, the government says, an earlier review of the arrangement would have been based on "inadequate experience".

according to Pearson, account for some of the departures. But there is some evidence that recent government initiatives, and the successes of the partly public company Celltech, have persuaded some British expatriates with important skills to seek to return to Britain.

The lower standard of living in Britain remains a problem. Few British companies in the field offer their scientists equity holdings, and the salaries of scientists on civil service pay scales (a significant proportion of those the study will cover) have fallen markedly behind those of their administrative equivalents since 1978.

A separate study initiated by CORDI, the EEC Committee on Research and Development, will look at the high level skill needed of the European semiconductor industry: in this area the exodus is predominantly from the industrial rather than the academic world. At the moment the recession is ensuring that demand in Britain remains at a low level, but, says Pearson, there is no room for complacency: an upturn in the economy could result in a shortage.

Tim Beardsley

High-energy physics

Researchers face risk of neutron shortage

THE European neutron beam community is facing a lean time. With applications for "beam-hours" $2\frac{1}{2}$ times the number available last year, the principal neutron source in Europe at the Institut Laue Langevin (ILL) in Grenoble is now off the air for three months, and will shut down for major repairs for at least a year beginning in August 1984. The result will be a major hiatus in neutron research programmes: although ILL officially involves only three countries (Britain, France and West Germany) the 300–400 British neutron researchers alone run around 1,000 experiments a year there, and groups from other countries join in collaborations.

In the face of this "neutron shortage", research groups are seeking neutron time elsewhere, mainly at Brookhaven, Oak Ridge and Los Alamos in the United States, where the facilities are intended largely for local users. According to one British neutron user, only "entrepreneurial types" and those with good American connections are likely to find many neutrons in 1984–85.

There are two European lights on the horizon, however. Britain's Spallation Neutron Source (SNS), under construction at the Rutherford and Appleton Laboratory in Oxfordshire and the French reactor Orphée. SNS will use an intense proton source to bombard a uranium target, producing many orders of magnitude more neutrons than ILL at high energies, a few fewer at intermediate (epithermal) energies and about the same number of low energy (cold) neutrons. Orphée is a thermal reactor, like ILL, but of about a third the power. The reactor has had teething troubles, but is just about to begin serious operation.

In Britain, SNS engineers have just demonstrated the linear injector, which accelerates hydrogen ions to 70 MeV, and the cyclotron sections, which will take protons to first 550 MeV, and later 800 MeV. This should be giving beam (and thus neutrons) by mid-1984, just before ILL's shutdown. However, the initial neutron intensity will be low, around one-tenth of the maximum, and it will take two years to wind up the intensity fully. This is because the proton beam is of very high intensity, and any slight mistuning of the accelerator could cause the beam to irradiate internal components to intolerably high levels. Thus intensity can be increased only as tuning improves over a couple of years.

Moreover, SNS will at the outset be short of funds — ILL has been a success primarily because it began with a full complement of high quality instruments (spectrometers and so on). SNS has room for 25 instruments, operating on 18 beam

lines, but it will begin with only six, with a possible seventh coming from India, where the Bhabha Atomic Research Centre has signed the only international agreement so far to work with Britain on SNS. Negotiations are also in progress with West Germany and Italy.

Moreover, at the Science and Engineering Research Council, which pays for SNS, there is a feeling that too much is being spent on neutron research.

Meanwhile, at 10-year-old ILL there are two problems: beam tubes have become embrittled through neutron exposure, and must be replaced; and a large, internal hole in a baffle plate in the reactor cooling circuit must be replaced. The French nuclear safety committee (SCIN) is considering how long ILL can continue without repairing the hole. By May this year, ILL staff hope to be given a "driving licence" to continue running with the hole unrepaired until autumn 1984. Then the repair has to be made. If it can be done through the top of the reactor, a year should be enough; if not, and the reactor has to be completely disassembled, it could be longer.

Robert Walgate

Polish science

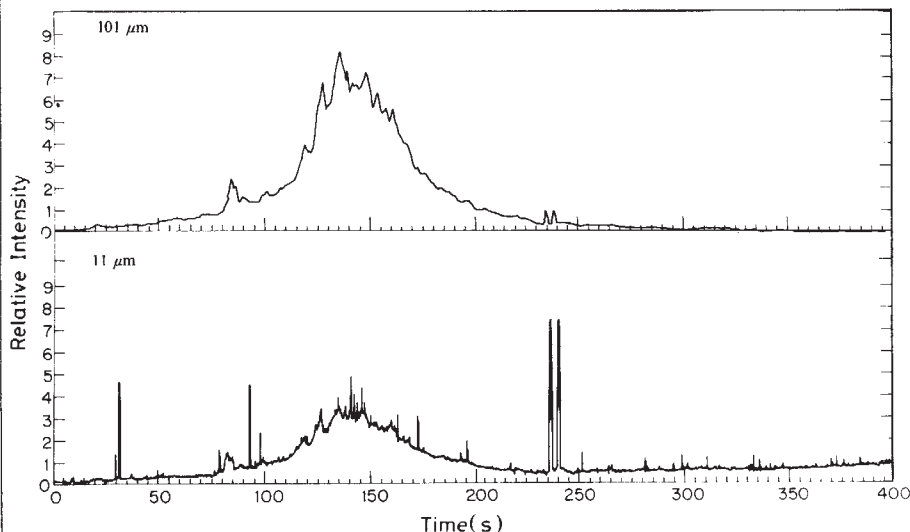
Talk of change

RESPONSIBILITY in Poland for applied science and its industrial applications should be transferred from the Academy of Sciences and the universities to a new state body at ministerial level, according to Dr Zdzisław Kaczmarek, chief academic secretary of the academy. Last month, Dr Kaczmarek told the plenary meeting of the academy that the universities and the academy should retain responsibility only for basic research and that the new body should be responsible for all industry-related research.

Such a policy would represent a total reversal of Polish science planning over the past ten years. Until 1972, Poland followed the usual Comecon model of having a "Committee of Science and Technology" at ministerial level. In that year, however, it was decided that, with 75 per cent of the research potential concentrated in the universities, the committee should be absorbed into a new Ministry of Science, Higher Education and Technology.

In 1977, at the Jablonna conference on science policy, the then academic secretary of the Polish Academy of Sciences, Dr Jan Kaczmarek (no relation), explained that the main advantage of the combined ministry was that it could increase

IRAS scans the Southern Cross



THE results of a scan of the Milky Way by the telescope of the Infrared Astronomy Satellite, launched on 26 January (see *Nature* 27 January, p.275). These data were obtained an hour after the protective cover of the telescope was removed. The scan covered 25 degrees of arc at 45° across the plane of our Galaxy in the constellation Crux, the Southern Cross.

The upper tracing is dominated by 100 μ m wavelength emission from cold dust associated with the material out of which all stars in the Galaxy are formed. The central bulge extends approximately 10° . The structure in the tracing is due to

individual clouds of dust and molecular gas hundreds of light years across. (The two spikes on the right side of the central peak are from an internal calibration source within the telescope and indicate the relative scales of the two graphs.)

The bottom tracing shows the intensity at a wavelength of 10 μ m and is mainly due to emission from thousands of millions of stars. The bulge at the centre shows the densest concentration of stars; the slow increase to the right of the scan is caused by warm dust in the plane of the Solar System. The individual narrow spikes are caused by single bright or nearby stars.

university participation in research. But he acknowledged that the combined ministry probably represented only a "transitory stage" in Polish science planning.

In practice, all science and higher learning in Poland (save a small sector of basic research accounting for no more than 10 per cent of the total science and higher education budget) had to be linked to a complex hierarchy of "problems" of the national economy. This led to researchers trying to attach their projects to as high ranking a problem as possible, and to administrative difficulties when institutes wanted to renew books or equipment used jointly by all researchers.

By autumn 1980, it was accepted that the "problems" scheme had become inoperable and should be replaced by a looser structure of applied research based on contracts between industry and the research institutes or universities. But liberalization brought two other major problems into the open — the favoured position of institutes controlled by other ministries in the competition for funds and the strict separation of the universities from the academy.

In this respect, Poland has been an exception to the pattern of most Comecon countries, including the Soviet Union, where researchers in academy institutes can and do hold university teaching posts. The Polish arrangement has, at times, proved advantageous to individual scholars. Thus some of those who lost their university posts during the 1968 purge on the grounds of political unreliability found work within the academy network, where they had no direct contact with students.

Both these issues were raised and sympathetically discussed at the Solidarity Congress in September 1981, with the reservation that Poland already had too many graduates chasing too few jobs so that combining the roles of academy researcher and university lecturer might exacerbate the situation.

A third problem, little discussed outside the academy itself, is that the academy's academic secretary, a post of ministerial rank, is ultimately responsible not to the academy but to the Prime Minister. This, it was hoped, would be remedied by new legislation, a first draft of which was due for discussion by the academy general meeting in December 1981.

That meeting was pre-empted by the declaration of martial law, since when nothing has been heard of the promised legislation. Last month, however, the journal *Rzeczpospolita*, which follows an orthodox political line, suggested that the new law on the academy should be drafted and passed as soon as possible on the grounds that such a law might help to break down mistrust between the academy and the authorities, inducing Polish scholars to end their current stance of "internal emigration" — a euphemism for non-cooperation with the authorities and the government.

Vera Rich

Ohio radiotelescope

Seeing stars or golf balls?

Washington

OHIO State University was abruptly informed last month that the land beneath its radio telescope — the fourth largest in the United States — is about to become the site of the Delaware Country Club's new golf course. University officials, who were apparently taken completely by surprise, are trying to work out a temporary arrangement with the country club, but there seems to be little chance that the telescope will be allowed to remain for more than five years.

Dr John Kraus, director of the radio observatory, calls the situation "a great scientific tragedy". An all-sky search for 21-cm wavelength sources, completed two years ago at the observatory, catalogued 20,000 objects, including many undiscovered quasars, some of them the most distant quasars yet seen. At present, the chief work of the observatory is its participation in the National Aeronautics and Space Administration (NASA) search for extraterrestrial intelligence.

Although the observatory was built by Ohio State University (with funds from the National Science Foundation), the land it sits on was provided by Ohio Wesleyan University. Under the terms of this joint venture, the universities were to share in the use of the observatory, but Ohio Wesleyan's interest has apparently waned in the 25 years since it was built. Last October, Ohio Wesleyan offered the country club an option to buy a 250-acre parcel and that includes the 100 acres currently leased by the club and 150 additional acres — among them the 15 acres occupied by the radio telescope.

According to Larry Thompson, assistant to the president of Ohio State, the university was told only after a contract for the sale had been signed.

A spokesman for Ohio Wesleyan says that its president, Thomas Wenzlau, had in fact discussed the sale of the land with the former provost of Ohio State two years ago, and that Ohio State "indicated they wanted to discontinue operation of the telescope by 1984".

"Ohio State showed no interest in

buying the property", he says. The spokesman suggested that the cause of the confusion may be the change in the Ohio State administration — both the provost and the president have left since that discussion took place. The spokesman adds that no Ohio Wesleyan faculty members now use the observatory and "we know of only one Ohio Wesleyan student who's used it in the last 10 years".

Ohio State officials have had one meeting with representatives of the country club to see if the telescope could remain. Earlin Harris, a member of the club who is involved in the negotiations, says, "I think all the issues will be resolved within a month of so".

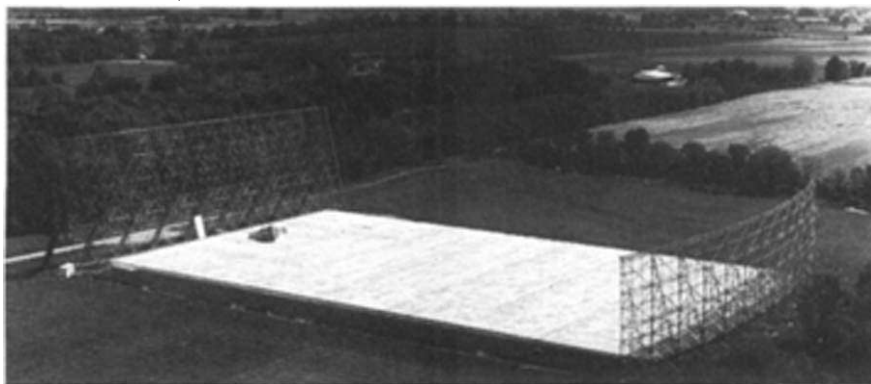
But the university does not expect a permanent solution. Dr Howard Sachs, associate provost at Ohio State, concedes that the university is in principle resigned to closing down the observatory. "The major problem is that we have only two faculty [members] right now who are intimately involved in its operation", he says. Kraus, the director, is one; he is a professor emeritus. The other is Dr Robert Dixon of the computer sciences department, who is not a full-time astronomer. The design of the telescope, which Kraus says provided the greatest aperture per unit cost, is tailored to the task of scanning the entire sky — making it of only limited use for most radioastronomy research.

Replacing the telescope would require an investment of \$5 million, according to Kraus; transporting it to a new location would be nearly as expensive. Sachs says that from the university's point of view, five years "would be a reasonable time" to try to keep the observatory open.

The country club has its own reasons for wanting to get rid of the observatory — and not just its plans to expand its nine-hole course to 18 holes. Sachs says the purchasers plan eventually to build residential units along the fairways. And the telescope, he admits, "is not the most desirable thing to have in your backyard. It looks like a couple of drive-in movie theaters that went out of business".

Stephen Budiansky

The threatened telescope



UK public spending

More money for industry

THE British government is putting its money where its mouth is. The annual estimates of public spending for the next three years, published last week*, record an increase of spending on industrial research and development next year by close on 40 per cent, from £158 million to £218 million. Most of the extra cash will go on the Department of Industry's programme of support for information technology, production engineering, computer-aided design, microelectronics and fibre optics.

These developments have been well advertised by government spokesmen in the past year, although the size of the budget increase is a surprise. But the total of £218 million has also to support laboratories run by the Department of Industry, the National Physical Laboratory, for example. On past form, the bulk of the extra money will be spent on grants to industrial companies.

Elsewhere in industrial research and development, the British government hopes to economize. While aerospace will get an extra £3 million (to a total of £33 million), the cost of space research and development, chiefly the Department of Industry's subscription to the European Space Agency, will fall by the same amount. It is also planned to save about £11 million (in a total this year of £263 million) on energy research.

Support for academic science is broadly that forecast some months ago. The research councils will have £436 million to spend in 1983-84, together with £81 million on capital projects. This budget includes the extra £4 million found for the British Antarctic Survey. The government document also says that the extra £10 million to be found for support of the natural sciences will not be available until 1984-85.

Those who look to the published spending plan for a forecast of how the universities will be dealt with after the present contraction will be disappointed. The capital expenditure of British universities is to be pegged at £120 million a year for the next two years (beginning on 1 April),

unchanged from this year and last. The recurrent budget of the University Grants Committee for 1983-84 represents an increase in cash terms by 5.2 per cent to £1,320 million, including a special supplement of £12 million (rising to £30 million in 1985-86) for new posts in universities.

The budget statement seems to accept that the "present phase of contraction" in higher education will not be complete by the beginning of 1984-85 (as originally intended for the universities) but only "during" that year.

For the rest, the annual expenditure statement is as usual a poor guide to what the government will be spending on science and technology. Provision for defence research will be known only when the Defence Estimates are published next month. Next year's research spending by the Department of the Environment is lumped together under "central and miscellaneous services" for which £113 million is allocated.

The statement does, however, vividly illustrate some of the British government's present preoccupations. It is, for example, planned that next year's capital investment of £6,904 million will require the government to supply £2,625 million, the bulk of this to the National Coal Board and British Rail. The electricity industry, on the other hand, is reckoned to contribute to this capital sum to the tune of £300 million (at electricity consumers' expense), while British Telecom is intended to finance a capital programme of more than £2,000 million out of current revenue.

Economically, this policy seems forced on the government by the obdurately high proportion of the Gross Domestic Product (GDP) consumed by public expenditure (23.5 + 0.5 per cent) in the past five years. In the still current financial year, the gross cost of servicing the national debt is estimated as 20 per cent of GDP, giving total public expenditure equal to 44 per cent of GDP for the year. □

**The Government's Expenditure Plans 1983-84 to 1985-86* (HMSO, Cmnd 8789-I & II, £5.10 & £9.75).

Recent and future spending on higher education (£ million)

Recurrent	1981-82	1982-83	1983-84	1984-85	1985-86
Universities	1,036	1,253	1,320		
Direct grant	82	95	97		
Polytechnics	487	547	560		
Student awards	891	762	769		
Sub-total	2,496	2,657	2,746	2,850	
Further education	813	892	877	890	
Adult	73	81	65	70	
Capital					
Universities	120	120	120	120	
Other	90	82	74	70	
Total	3,592	3,832	3,882	4,000	4,150

US national laboratories

Materials centre plan from LBL

Washington

THE Lawrence Berkeley Laboratory (LBL) in California last week entered the contest for the national laboratory with the most indispensable role by unveiling plans for a \$138 million National Center for Advanced Materials (NCAM). The centre, due to open its doors in 1989 if Congress approves a 1984 start on construction, represents a change of direction for LBL. According to the laboratory's director, David Shirley, the centre would eventually involve one-quarter of LBL's staff of 2,500 full-time employees and \$30 million of its \$140 million annual operating budget.

Initiatives such as NCAM have become more frequent as the role of the national laboratories has come under increased scrutiny by Congress and the Administration. The national laboratories' efforts to develop indispensable functions have at times reached absurd proportions, as in the recent fanfare that accompanied Argonne National Laboratory's establishment of a national centre for coal reference samples.

LBL's plan, however, comes with a strong endorsement from White House science adviser George Keyworth, and it is a centrepiece of the proposed 1984 budget for the Department of Energy (DoE) general sciences programme. Congress is being asked to approve \$34 million with which to begin construction this autumn. The heart of the new centre will be a "next-generation" synchrotron radiation source, associated with which will be three laboratories, covering advanced materials synthesis, surface science and catalysis and advanced device concepts.

Although most of the operating funds for the centre will come from DoE, Shirley stresses the importance of close collaboration with industry. Shirley says the goal of NCAM will be to conduct basic research in fields "just on the horizon for America's high-tech industry".

While acknowledging that LBL's shift to materials science will entail phasing out other research areas, Shirley said that the laboratory's highly-respected high-energy physics research would be spared.

Shirley says he believes that the NCAM proposal will be well received in Congress, particularly as it addresses "institutional problems" that Congress has been concerned about. "We hear a lot about how Japan and Europe are getting ahead of us in high-tech areas", he says, "and we hear a lot about the reasons, too — that their industry, universities and national labs work together". Noting the close ties LBL already has with the University of California, Shirley says NCAM is a serious effort to do the same in the United States.

Stephen Budiansky

Soviet Antarctica

When will the miners move south?

LAST month's meeting in Wellington, New Zealand, of representatives of the fourteen signatory states of the Antarctic Treaty reported "good progress" towards the establishment of an authority to control future commercial activity in Antarctica. But some of the most important problems have yet to be solved. According to Mr Chris Beeby, chairman of the New Zealand meeting, the greatest difficulty is that of reconciling the positions of the seven states which claim sovereignty over some sector of the Antarctic both among themselves and with those which do not. Some spirited clashes must be expected on the way to a final formula before the treaty falls due for renewal in 1991.

Before then, an agreement will also have to be reached on the exploitation of mineral resources in Antarctica, on which the treaty as it stands says nothing. Although the exploitation of Antarctic marine resources, krill for example, is regulated by a convention signed in 1980, the simple extension of this precedent to the regulation of mineral exploitation of land will be complicated by territorial claims but also by arguments such as those which dogged the Law of the Sea negotiations.

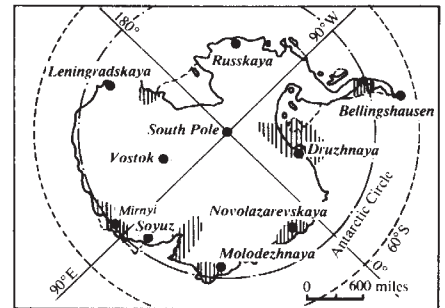
Neither the Soviet Union nor the United States claims territorial rights, but the Soviet delegation at the Law of the Sea negotiations consistently opposed US views on seabed mining beyond the continental shelf on the grounds that the "capitalists" were seeking to stake their claims before the developing countries had evolved the necessary technology. Similar reasoning is likely to dictate the Soviet stance on Antarctica. If seeking to become the guardian of the developing countries, by espousing the status of Antarctica as part of the common heritage, however, the Soviet Union will face competition from within the developing world now that India has become so active in Antarctic affairs (see *Nature* 3 February, p.362).

The Soviet Union was a relative latecomer to Antarctic exploration, although as successor to the tsarist empire, it can claim a link with the first definite

sighting of the Antarctic continent, by Bellingshausen and Lazarev in 1821. The first Soviet expedition was not despatched until 1956, but for the past 27 years, the Soviet Union has maintained a permanent presence in Antarctica. It now has seven permanent bases (see map), supplemented by occasional "summer" bases. The latest of these, Soyuz, was established last December in an area which is of "particular geological interest", according to Dr Garick Grikurov, one of the main organizers of the Soviet Antarctic programme. Dr Grikurov has said that the area contains promising deposits of commercially important minerals.

During the past 25 years, Soviet teams have mapped and carried out geological surveys of a sizeable area of Antarctica, paying particular attention to the "oases" — regions of ice-free rock. But the large size of the Soviet expeditions — the present one has a staff of more than 1,000, although this may include the crews of the five research and support ships and aircraft pilots — means that the mineralogical

surveys are only one part of a broad research spectrum. One major project due to be completed this year is a deep drilling experiment designed to take ice cores from a site near the Vostok icecap station down to bedrock (a depth of some 3,500 m).



Areas where the Soviet Union has carried out detailed geological surveys

Although the Soviet emphasis remains scientific, it is unlikely that Soviet planners are blind to the commercial possibilities of their mineralogical and geological findings. And when the time comes for the commercial exploitation of Antarctica, experience gained in mining in Siberia and the far north should give the Soviet Union a flying start.

Vera Rich

Sizewell B inquiry

Appeal fund swelled by dignitaries

THE Sizewell B Appeal Fund, set up in a flurry of activity only three weeks ago, now has seven distinguished trustees to oversee applications for financial support from objectors at the public inquiry now taking place at Snape in Suffolk into the proposal to build a pressurised water reactor at Sizewell, also in Suffolk. Among the trustees are Professor Sir Hans Kornberg, master of Christ's College, Cambridge, and Professor Paul Matthews, Vice-Chancellor of the University of Bath.

The Appeal Committee has set itself the task of raising "at least" £0.5 million to support objectors at the inquiry. Bitter protests followed the decision in September by Energy Secretary Nigel Lawson not to allow the use of government funds to support objectors to the plans of the Central Electricity Generating Board (CEGB) to build a reactor at Sizewell, and some groups were forced to drop their case. On the first day of the inquiry, CEGB chairman Sir Walter Marshall, during a remarkable spontaneous walk-about debate with demonstrating protesters, said he would be "delighted" if a public appeal to fund the objectors succeeded in raising money.

Mr Edward Irving, a barrister and a Suffolk county councillor, took up the gauntlet, and on the 15 January Lord Kearton, former chairman of Courtaulds and a Fellow of the Royal Society, agreed to act as a trustee. Since then, some of the trustees have made it clear that they support CEGB's case, but feel the chronic



lack of funds for the objectors is a denial of natural justice. CEGB has already spent an estimated £5 million in preparing its case.

Mr Irving admits that, with the inquiry already under way, it is probably rather late in the day to start raising funds for the objectors, but he is confident that although no further basic research will be possible, the fund will enable protesters to raise some additional points. Professor Kornberg pointed out that at no recent important public inquiry had objectors won the day, and that the massive imbalance of funds between the sides could have had something to do with it. There is also the hope that a better organization for the objectors could actually speed up the inquiry — which is likely to last for at least six months.

Tim Beardsley

Oil search denied

SOME consternation was caused at the Wellington meeting by the news that Sir Peter Scott son of explorer Captain Robert Scott, and a naturalist, had accused the governments of Australia, France, Japan and West Germany by telex of making plans to search for oil on the Antarctic shelf, a charge hotly denied by the government of Australia. Scott said on the telephone last week that his information had come from a confidential source, and that he was "delighted" to be told that it was wrong. □

Nuclear reactors in space

One has come down, but more are still to come

Washington

SPACE-BASED nuclear reactors — like that carried by the Soviet Cosmos 1402 satellite, which has just made an unscheduled re-entry into the Earth's atmosphere — are becoming an increasingly important part of US military preparations for war in space.

At present, only the Soviet Union is putting nuclear reactors into Earth orbit. But now the United States, having lost interest after putting up a single reactor in 1965, is back at work again. Two separate projects are under way to design a reactor with a lifetime of seven years that will yield 100 kW of electricity. A working US reactor could be in orbit by the early 1990s.

Behind the renewed US interest are three separate military goals. The first, and apparently the most important to defence planners, is "survivability" of critical surveillance and communications satellites in time of war. "A reactor is hardenable", said a Department of Energy (DoE) source, "solar is not". Solar panels provide a large and vulnerable target for a Soviet anti-satellite (ASAT) weapon. Defense Department planners are increasingly concerned about the Soviet Union's crude but functional ASAT, which can disable a satellite simply by exploding. The Defense Department view is that a nuclear-powered satellite would also be considerably more manoeuvrable than one equipped with cumbersome and fragile solar panels.

The second goal is the development of radar ocean surveillance satellites, similar to Cosmos 1402. According to Rear Admiral Eugene Carroll, USN (Ret.), now deputy director of the Center for Defense Information in Washington, the Soviets use these satellites to target the US fleet for attack by SS-11 ground-based inter-continental ballistic missiles. The high power requirements of the radar, and possibly the Soviet Union's inferior solar technology, require the use of a nuclear power source. The Cosmos 1402 reactor reportedly generates 100 kW thermal with an electric output in the range 5–20 kW.

The power requirements of US satellites may be even greater. They will probably be designed for higher resolution so as to pick up the smaller ships of the Soviet fleet (which has no giant aircraft carriers), while US radar satellites would probably be put in orbits higher than that of 250 km chosen for Cosmos 1402 so as to escape ASAT attack and for longevity. Soviet practice has been to plan for a lifetime of only a few months and then to remove the nuclear reactor from the Earth's atmosphere by boosting the satellite to a higher orbit.

"As you move towards radar in space, you can make a case for 10 or 100 kilowatts", says Herbert York, a former

Director of Defense Research and Engineering. And at the current price of solar cells, that is just the power range at which nuclear power begins to be cheaper than solar, although the exact breakpoint seems to depend on the ideology of the observer. The largest solar array the United States is planning is a 25-kW unit to be launched on a forthcoming shuttle flight.

The third goal, and perhaps the least significant at this stage, is the production of exotic laser and particle-beam weapons. These would need vast amounts of power that only a nuclear reactor could supply.

The renewed interest in space-based reactors has set off a row between DoE on the one hand and the National Aeronautics and Space Administration (NASA) and the Defense Advanced Research Projects Agency (DARPA) on the other. DoE is already working on a 100-kW reactor design at its Los Alamos National Laboratory.

By law, DoE has authority over all nuclear programmes in the government, a result of the early decision to maintain civilian control over nuclear activities, including weapons research and construction. Nevertheless, NASA and DARPA have joined together and recently circulated a request for contractors to design a 100-kW space reactor. A NASA official said the request had drawn "a lot of interest" from the industry. Proposals were due at the end of January, and the selection of a contractor is to be made shortly. The design requirements are classified.

The military's intentions seem, however, to be a good deal fuzzier than their interest would indicate. According to Dan Deudney of the Worldwatch Institute, plans for a radar surveillance satellite have not gone beyond the drawing board; an earlier design, known as "Clipper Bow",

Cosmos 1402 safe

THE nuclear fuel core of the ill-fated Cosmos 1402 apparently burned up safely over the South Atlantic last Monday, although there was some doubt about the exact point of destruction, since the reactor fragment was tumbling wildly during its final moments.

A satellite of similar type, Cosmos 1412, carrying larger radar systems to monitor naval movements, is reported by French experts to have concluded its operations at the end of January. In this case, however, the reactor was successfully disconnected from the main spacecraft and boosted into a high parking orbit with a perigee of 901 km.

Vera Rich

was abandoned not long ago. A NASA official involved with the NASA/DARPA project acknowledged, "We really don't know yet what the applications are".

Meanwhile, more criticisms over the deployment of nuclear reactors in space are cropping up, and not just because of the dangers exemplified by the Cosmos 1402 accident or the 1978 crash of the similar Cosmos 954 in Canada.

Dr Robert Bowman, who as director of Advanced Space Programs Development for the US Air Force twice cancelled the military space reactor programme, argued at a Washington press conference last month that "reactors have vulnerabilities of their own" with respect to ASAT attack. Bowman, who has left the Air Force, said that the low efficiencies of nuclear reactors — typically 5 per cent — mean that large heat radiators are required to dissipate the excess heat. These radiators "stick out like a sore thumb in the infrared", he said.

But the critics seem chiefly concerned that space-based reactors open the door for uncontrolled escalation of the arms race in space. "We can do all the verification and monitoring we need to with photovoltaics", says Deudney, "but we can't fight a war with photovoltaics." And Admiral Carroll sees a link between nuclear reactors and the ASAT arms race: "Absent an ASAT threat, there is no reason why higher space power requirements cannot be met by larger solar arrays."

Negotiations on an ASAT ban were suspended by President Carter in 1980 in retaliation for the Soviet intrusion into Afghanistan, and have not been resumed. The United States is developing a "direct ascent" ASAT that will be fired by the F-15 fighter plane. Although it will have an altitude range limited to about 1,000 km, it will — unlike the ground-launched Soviet system — be able to hit satellites in any orbital inclination. And Carroll warns that once it is deployed, it could make agreement on an ASAT treaty almost impossible by drastically increasing the difficulty of verification. The ASATs would not be readily distinguishable from other F-15 armaments; and on-site inspections of F-15 bases would be out of the question. The ASAT is due to be flight-tested this spring, with deployment in 1987. "Time is running all too short", Carroll said.

For at least the next decade, US military satellites will continue to be solar-powered; the military has shown little interest in further use of the relatively inefficient radioisotope thermoelectric generators (RTGs), which are used to power planetary missions and which have been used for military satellites such as the Transit navigation system. These devices, which are not reactors, use the heat from the radioactive decay of plutonium to generate electricity. Future US use of nuclear reactors in space, however, may well hinge on the progress made in controlling the arms race in space.

Stephen Budiansky

New accelerators

Delay at Geneva

If you win some, you can afford to lose some. That seems to be the philosophy at CERN, the European centre for nuclear physics near Geneva. With a few intermediate vector bosons in the bag (see *Nature* 27 January, p.285), the laboratory seems now stoically to have accepted that its next big accelerator, LEP, will be a year late.

For years there has been pressure on CERN to build LEP quickly, and ahead of the competition in the United States. CERN director-general Professor Herwig Schopper last week told senior staff that because of problems in the approval procedure, LEP is likely to be delayed by six months to a year.

LEP will be a giant underground ring, 27 km in circumference, of magnets and vacuum pipe designed to collide multiple bunches of electrons and positrons into one another at sufficient energy, intensity and frequency to produce copious numbers of intermediate vector bosons and other particles. The immediate competitor, the Stanford Linear Collider (SLC), has similar objectives but different means: electrons and positrons in single ultra-intense and narrow bunches produced by the Stanford Linear Accelerator. These bunches will collide only once.

SLC is smaller and cheaper than LEP. According to Nobel prizewinner Burton Richter who is designing SLC, it will produce collisions in autumn 1986. LEP will not be ready for experiments until late 1988. But CERN staff expect SLC to face many technical difficulties. Although designed to produce as many as a quarter of the number of intermediate vector bosons as LEP, SLC may do little more than repeat the current low vector boson production rate at the CERN proton-antiproton collider.

Meanwhile, there is friction between the village of Echevenex, which has a major access pit rising in a local beauty spot overlooking the village, and CERN management. Villagers are not satisfied that the horizontal tunnel they requested would cost SF 27 million (£8.6 million); their advisers say SF 15-17 million, compared with the total LEP cost of around SF 1,000 million (£320 million). But the environmental commissioners accepted CERN's case that the tunnel was too expensive. The villagers are now attempting to block the approval procedure. Ultimately the prefect of the region can overrule them, but this takes time.

So, instead of final approval coming from the French constitutional court in the spring, as expected, it might not arrive before end of the year. But the drilling of the main LEP tunnel is the most time-consuming part of LEP construction, so if the starting time is delayed, so is LEP.

Robert Walgate

US science travel

Fulbright helps

Washington

A CHANGE in the migratory pattern of US scientists seems to be discernible, but it is too soon to tell what it means. After some years of a falling trend of overseas visits by US scientists and engineers, applications for the Fulbright travel funds are now booming. So varied are the purposes of foreign travel, and the sources of funds therefor, that looking for national trends in the Fulbright data may be no better than reading tea-leaves. These, however, are the facts:

- Applications by and awards to graduate students for study in Western Europe have increased in the past three years. The Institute of International Education in New York, which administers the graduate student programme, says that awards increased from 30 in 1980-81 to 35 last year and 45 in the present academic year. Half of the students mainly in chemistry and biochemistry, opt for West Germany, with Switzerland ranking second.

- Applications for Fulbright travel and research awards from scholars seeking to visit France are dramatically up. The Council on International Education

(responsible for this programme) says that it has already received 70 applications for visits to France, more than the number of awards last year.

- The United Kingdom remains one of the most popular destinations for US scholars, so that even though awards amount to only \$500 a month, only one in ten applications succeeds. The Council for International Exchange of scholars in Washington says that applications from scientists, especially biologists, are increasing. Graduate students face even longer odds, with 600 applications for the 30 Fulbright places awarded each year in the United Kingdom.

Those close to these programmes say that the changes which have taken place may be only blips on the chart. Interpretation of the data is in any case complicated by the way in which the Fulbright programmes vary from country to country: thus Austria picks only eight scholars, mostly scientists, but West Germany encourages scientists to apply for Humboldt Fellowships instead. Dr Dorothy S. Zinberg (Harvard), a student of international scientific travel (see *Nature* 295, 605; 1982) says that a more systematic study of the trends is needed, and that people may be applying for Fulbright funds because other sources have dried up.

Deborah Shapley

Nature index of biotechnology stocks

12-month High	12-month Low	Company	Close previous month	Close 28 Jan.	Change
53*	16 1/8	A.B. Fortia (Sweden)	44 7/8	49	+ 4 1/8
8	2	Bio-Logicals (Canada)	4 1/4	3 3/4	- 1/2
10 3/4*	3 3/8	Bio-Response (USA)	7 3/8	10 3/4*	+ 3 1/8
14 3/8*	7 3/4	Cetus (USA)	12 3/8	14 3/8*	+ 2
12 3/8*	6 1/8	Collaborative Research (USA)	11 7/8	12 3/8*	+ 3/8
20*	5 3/4	Damon (USA)	16 1/8	19 3/4	+ 3 3/8
28*	8 1/4	Enzo-Biochem (USA)	26 1/2	28*	+ 1 1/2
28	6 5/8	Flow General (USA)	11 7/8	14 3/4	+ 2 7/8
53 3/4*	26	Genentech (USA)	41 3/4	53 3/4*	+ 12
12 1/2*	2 1/4	Genetic Systems (USA)	8 1/2	11	+ 2 1/2
17 3/8*	7	Genex (USA)	12 7/8	16 3/4	+ 3 7/8
27 3/4	9 3/8	Hybritech (USA)	22 1/4	26 1/4	+ 4
16 3/4*	5	Molecular Genetics (USA)	14 3/4	14	- 3/4
52	34 7/8	Novo Industri A/S (Denmark)	43 7/8	49 3/4	+ 5 7/8
27	8	Monoclonal Antibodies (USA)	20 1/2	19 1/2	- 1

The *Nature* Biotechnology Index for January 1983, stands at 181.7 compared with 157.5 for December 1982. Base is 100 as of 25 June 1982. The previous index appeared in *Nature* 13 January, p.105. Where stocks are traded over the counter, the price quoted is the bid price. Where stocks are traded on the American and New York stock exchanges, the price quoted is the transaction price. Yearly highs and lows are based on Friday closing prices.

*New high or low price.

In January, *Nature's* index of biotechnology stocks scored its biggest single gain since its formation last June. The follow on rise reflects the continuing interest of large investors such as institutional endowment funds and the decline of the US dollar in January against the Swedish and Danish kroner. This made the stock of the two largest companies in the *Nature* index, A.B. Fortia and Denmark's Novo Industri, especially attractive to US investors.

Another factor was Genentech's stock, which moved upwards sharply in mid-January after the company held a meeting for a hundred securities analysts at its headquarters in south San Francisco. Smaller companies have also been doing well in the stock market lately; the stock of Enzo-Biochem of New York, which traded at around 14 before the market surged in October, closed at 28 on 28 January.

Deborah Shapley

Thalassaemia

SIR — Orkin et al. in *Nature* of 23/30 December 1982¹ elegantly demonstrated that decreased synthesis of mutant globin β^E chains is due to abnormal processing of the β -specific mRNA primary transcript. This explains the previous finding of decreased mature mRNA in Hb E reticulocytes². Thus, a qualitative change in globin structure is associated with a quantitative change in its production. This is a vindication of the "structure-rate" hypothesis proposed by Itano a quarter of a century ago³ and further elaborated by Ingram and Stretton⁴. At that time it was suggested that an electrophoretically silent or a synonymous codon replacement might be present in β -globin in β -thalassaemia. If this entailed the requirement for a tRNA species absent or scarce in erythroid cells, it would explain why the polypeptide chain was synthesized at a reduced rate⁵.

Apart from the special case of Hb Lepore, the search for a structural abnormality in the β -globin chain in β^+ -thalassaemia has since failed. However, we now have a clear example of a β -globin chain which was already known to be structurally abnormal, and in which a reduced rate of synthesis (β^+ -thalassaemia) is indeed a direct result of the structural abnormality. It turns out that the link between structure and rate is not at the stage of translation, but rather at the earlier stage of processing of the primary transcript. The very existence of this was of course totally unknown at the time when the structure-rate hypothesis was originally formulated.

LUCIO LUZZATTO

Royal Postgraduate Medical School,
London W12, UK

1. Orkin, S.H. et al. *Nature* **300**, 768-769 (1982).
2. Traeger, J., Wood, W.G., Clegg, J.B., Weatherall, D.J. & Wasi, P. *Nature* **288**, 497-499 (1980).
3. Itano, H. *Adv. Protein Chem.* **12**, 215 (1957).
4. Ingram, V.H. & Stretton, A.O.W. *Nature* **184**, 1903 (1959).
5. Itano, H. *Symposium on Abnormal Haemoglobins in Africa* (Blackwell, Oxford, 1965).

More Chinese names

SIR — I agree with S. Jellis (*Nature* 9 December 1982, p.476) that it is now common in journals from Mainland China to print the transliterated given names as one word. It is also true that usually the surnames have one syllable and the given names have two syllables. Unfortunately it is by no means rare to find two-syllable surnames or single-syllable given names. Perhaps the editors should communicate with the authors to confirm their surnames when in doubt — for instance, when a name such as Ouyang Fan appears. (Both Ouyang and Fan can be Chinese surnames. In this particular case, the gentleman I know is Mr Ouyang).

CHEN-TUNG A. CHEN

Oregon State University,
Corvallis, Oregon, USA

Ball lightning — all in the mind?

SIR — There may be a physiological explanation for the lightning-associated phenomenon reported by Burbidge and Robertson in the 16 December 1982 issue of *Nature* (p.623).

On looking at a bright light an after-image is produced. If the light consists of consecutive streaks of lightning running in different directions then the after-image may include a blob where subsequent flashes cross the after-image of previous flashes. The final after-image may well appear like a "central body" with "arms" flowing from it. As the subject moved her eyes, so the after-image would move in respect to the surroundings. Because the image was appearing to move, the subject's eyes would attempt to follow it which would cause the image to move more. As soon as the subject fixed her gaze on an object (the pile of tools) the image would stop moving.

An after-image gradually fades away. The blob, being due to greater intensity of light, would persist longer than the arms and this would give the impression of the arms going back into the blob and the blob itself then disappearing.

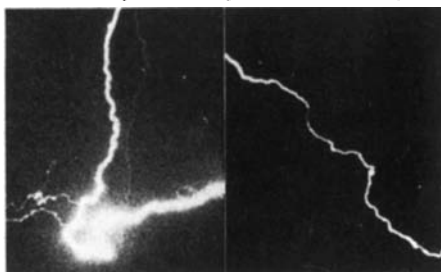
MAURICE SUTTON

Haringey District General Hospital,
London N18, UK

SIR — There seems to be some confusion over the use of the term "ball lightning" in recent discussions in *Nature*, for more than one phenomenon is involved.

(1) Relatively slow-moving luminous spheres may float across one's field of vision after a near lightning strike — probably electrostatically charged gas globules. The term ball lightning here is surely a misnomer, for the phenomenon is clearly a discharge sequela.

(2) Ball-shaped lightning itself is occasionally described, almost invariably by recipients of lightning strikes. To such the discharge, if seen at all, would be viewed along its longitudinal axis, and seen, therefore, as a fireball. Similarly, to



Left: Photograph of part of a lightning discharge showing as single puff. It is suggested that this, and those seen on the right are caused by the tortuous course of the discharge momentarily approaching (or receding from) the camera lens, and accordingly being seen along its longitudinal axis. **Right:** Photograph of discharge that has undergone a 90° change of direction. Several puffs are seen in the region of the "U" bend. To an observer at the receiving end of the horizontal component, the discharge would appear as a vertical streak ending in an expanding fireball. Photos by H.G. du P. Gillett.

an observer stationed at the receiving end of the main horizontal component in the right hand photograph, the entire discharge would appear at first as the familiar vertical shaft, but would end in a fireball.

(3) Conversely, to an observer placed diametrically opposite, it would appear very different. The vertical shaft would end as a relatively slow-moving, fading ball of light — perhaps the explanation for a reliably reported observation of an otherwise normal lightning streak that ended thus.

In the photographs, the puffs could be caused by the momentary observation of the discharges along their longitudinal axes, the amount of light being thus enhanced; the lightning's essential tortuosity increasing the puff's diameter.

H.G. du P. GILLET

Harrow, UK

Retroposons defined

SIR — Several classes of dispersed repeated sequences in mammalian DNA share common properties which imply that they are derived from RNA¹⁻⁴. Thus after a decade of speculation, "reverse" flow of genetic information is becoming established as a major contributor to the structure, if perhaps not the function, of mammalian genomes. The sequences concerned are the processed pseudogenes representing mRNAs¹, the pseudogenes representing small nuclear RNAs², and the Alu and related sequences representing various polymerase III RNAs³. In each class the RNA-related sequence is tailed by an adenine-rich segment and flanked by a 7- to 21-base pair duplication of the insertion site, implying a common mechanism of insertion. These traces of insertion differ from those of the other major type of dispersed repeated sequence, the transposons, which in contrast move by DNA transposition.

A new name seems to be required for the RNA-derived type of sequence, and I suggest "retroposons", to denote their RNA origin and their dispersed positions. Retroposons are present in several mammalian orders, and given one apparent retroposon in *Drosophila*⁵ they may be a feature of eukaryotes in general.

JOHN ROGERS

MRC Laboratory of
Molecular Biology,
Cambridge, UK

1. Hollis, G.F. et al. *Nature* **296**, 321-325 (1982).
2. Van Arsdell, S.W. et al. *Cell* **26**, 11-17 (1981).
3. Jagadeeswaran, P., Forget, B.G. & Weissman, S.M. *Cell* **26**, 141-142 (1981).
4. Sharp, P.A. *Nature* (in the press).
5. Dawid, I.B. et al. *Cell* **25**, 399-408 (1981).

Oh, brother . . .

SIR — Orwell's big brother we know about, but Aldous Huxley's . . . ? Surely your leading article of 27 January (p.271) can't be an attack on Julian? D SOWBY

ICRP, Sutton, UK

Coming to terms with Samoa?

Social anthropologists seem bent on having a row among themselves about the late Margaret Mead's monumental study. And not before time. But anthropology as such need not be damaged.

Samoa is not a place but a collection of islands and, worse still, is in the western Pacific. That is the first truth to acknowledge about the latest quarrel to divide the social anthropologists: hardly anybody has been there or can expect to be carried there by chance, as were the early Polynesians. There are, however, two notable visitors to the place — the late Ms Margaret Mead, who wrote a book called *Coming of Age in Samoa*, and now (his book is not yet published but merely publicized in advance) Dr Derek Freeman, emeritus professor of anthropology at the Australian National University, Canberra, Ms Mead set out to change the world, and more or less succeeded. Professor Freeman would change it back again, but only time will tell whether he can manage that.

Ms Mead was twenty-three when she first reached the island of Tau, in the Manu'a Archipelago. A student, even a protégée of Franz Boas, one of those who had the flair to recognize that there is a problem called social anthropology and the daring to suppose that cultural differences are culturally determined, Ms Mead reached Samoa more than half a century ago in a revolutionary state of mind. She had no ambition to change Samoa, but she sought desperately to illuminate, perhaps even to reform, her own society in the United States by what she had to say. To those who knew her, Ms Mead was always something of a paradox — always persuaded that when the facts were discovered, they would bolster up what she knew to be true.

By all accounts, Professor Freeman has followed the unkindest course and taken Ms Mead at her own valuation, arguing that it is false. In due course, his book will be formally reviewed, when it will be possible for readers who have not read it to tell what to make of it; meanwhile, Freeman's pulling of rank on Mead is telling; he claims to have been adopted as a stepson by a Samoan chief, and complains that Mead never lived in a leaky Samoan hut. Worse still, and much more telling, Freeman says that Mead misinterpreted what she was told by the Samoan adolescent girls whom she interrogated. Not merely, he says, did the experiment interfere with its subject, but it was part of it. Ms Mead was told what she said (in her broken Polynesian) she wanted to be told.

Ms Mead is dead, while Professor Freeman (emeritus already?) is no chicken. So why not let the quarrel rest? Freeman's

answer would be that for social anthropologists, the issue is too important. So it is for the rest of us. For whatever she may have intended on her first visit to Samoa, Margaret Mead's account has had a disastrous influence. Its simple message is that that are other societies, so well described that they can only be supposed to be *better* societies, in which adolescent girls live by their libidos, magically hardly ever become pregnant and nevertheless mature to become members of adult society driven by a sense of ritual. Rousseau's noble savage is by comparison merely a second-class citizen.

Freeman's case is strong. After half a century of hard work by social anthropologists, it is clear that Ms Mead's account of Samoa in the 1920s is incomplete even if not downright wrong. The index to *Coming of Age in Samoa* refers to funeral ceremonies only on the last page of the text, where it is in passing mentioned that people cut down plantations when about to bury their dead. Which modern social anthropologist would visit an uncharted place and fail to check the box on his questionnaire dealing with the disposal of the dead? Instead, Ms Mead half a century and more ago seems to have concentrated on the sexual proclivities of a score of teenage girls. In doing so, she broke new ground in social anthropology, and afterwards helped to change the sexual mores of the society from which she came. That she may have had no compelling evidence for her position in either of these intellectual revolutions is beside the point; Margaret Mead was so often sure that she was right, and so able to persuade others of the truth as she saw it, that the need for evidence must have been an encumbrance.

Several lines of evidence bear out this hard judgement, the chief of which is her own opinion. The first half-sentence of the narrative of *Coming of Age in Samoa* is "The life of the day begins at dawn . . .". When else, anywhere? Then, of Samoa, "under historical conditions very different from those which made Greece and Rome flourish and fall, groups of human beings have worked out patterns of life so different from our own that we cannot venture any guess that they would have arrived at our solutions". Really, even if the conditional is removed? And finally, "Samoa knows one way of life and teaches it to her children: will we, who have the knowledge of many ways, leave our

children free to choose among them?"

Among social anthropologists, Ms Mead's position has always been clear. A disciple of Boas, she was always an out-and-out culturalist, equally impatient with physiology and psychology and especially so with Freud. In her heyday, in the 1920s, Mead's jejune glimpse of Samoa (she was there for nine months on her first visit) had much to teach her contemporaries. But the professional wars that she was fighting have long since ended in professional compromise — each side's acknowledgement that its standpoint has been too narrow. If Professor Freeman's book tells us no more (and we shall have to wait and see) it will be a disappointment.

By all accounts, Freeman has other fish to fry. While his criticisms of Mead's work in Samoa are largely professional and methodological, the driving force for his scholarship seems to be resentment of Mead's easy success in changing the society in which she lived. In this respect, Freeman may give social anthropology, his own discipline, too little credit. For who is to say at this late stage that Mead was less a prisoner of the society from which she embarked to Samoa in the 1920s than the Samoans themselves? A child of Prohibition (and its attendant violence), it is natural that she should have found tranquillity in the south-west Pacific. With the Depression apparently cured by the New Deal, it is natural that she should have afterwards preached permissiveness (Samoan style), espoused liberal causes such as feminism and the banning of the bomb and have become a *guru* for the young (and so, by extension, somewhat intolerant of adults). But why so? The explanation is less likely to be found in Samoa than on Mead's native heath.

For anthropologists as such, the issue that has blown up around Ms Mead's original investigation in Samoa, and her popular account of it, is probably unimportant. While techniques have enormously improved in the past half century, it is even more important that people working in the field have learned how many are the pitfalls that confront them, and to the discovery of which Ms Mead has unwittingly made powerful contributions. Her agenda was correct: "a knowledge of one other culture should sharpen our ability to scrutinise more steadily . . . our own". She may have gone too far by asking that it should then be possible "to judge".

Plant pollination

Beetle vectors

from Peter D. Moore

THE reward most frequently offered to a potential insect pollinator by a flower is that of an available food resource, though there are many variations on this theme^{1,2}. Some flowers encourage visitors by possessing parabolic forms that focus solar radiation in their centres; others avoid the energy expenditure and pollen losses involved in the provision of free meals for pollinators by resorting to deceptive techniques, the most extreme of which is the offer of fake pollen in the form of yellow hairs on the lips of some orchids³. Such strategies are regarded as evolutionary advancements, but also entail a degree of risk if the foraging vector were to learn that its food-seeking efforts are in vain. Flowers that rely specifically on beetles as pollination vectors have evolved a variety of enticements for their insect visitors, ranging from the more usual food source to something more exotic — a concealed boudoir for mating activities.

The involvement of beetles as pollinators is in itself intriguing for, although this group of insects is not of great significance for pollination in moist temperate regions, it is much more important in semi-arid areas⁴ and in tropical regions¹. The antiquity of the beetles as a group and their frequent association with primitive flowers has led to the suggestion that beetle pollination represents one of the earliest types of insect vector pollination in flowering plants⁵.

An interesting example is provided by *Calycanthus occidentalis*, a shrub found along streams and in shaded gullies in California. Its attractive dark red flowers are considered to be primitive, in an evolutionary sense, and consist of a spirally arranged series of bracts and petaloid structures (tepals) that merge gradually into one another and eventually lead into stamens and carpels. In many ways the flowers resemble those of *Magnolia*, but they differ in that instead of the parts being arranged on a conical receptacle, they form a hollow cup, with the carpels seated at its base. Grant⁶ listed a number of insects, such as bees and flies, which visited these flowers, and regarded one particular beetle, *Colopterus truncatus*, as critical for pollination. Attracted to the flower by scent, the beetle enters the developing flower only to find itself trapped by the inward-pointing bracts and tepals which are equipped with a series of stiff bristles. As many as ten beetles may be trapped in a single flower and are forced to remain there, feeding on various floral parts. They become covered by sticky pollen when, a little later, the anthers dehisce but their release does not come until the opening of the flower itself.

The food available to the beetles while they are trapped in the flower comes from specialized cells which accumulate rich resources of proteins in vacuoles⁷. The existence of such highly specialized tissues indicates close co-evolution between the host plant and its pollinator.

A rather more advanced, subtle and energy-conserving technique for recruiting the services of beetles in the transport of pollen has recently been described by Beach⁸, working in the rain forests of Costa Rica on a rhizomatous shrub, *Cyclanthus bipartitus* (Cyclanthaceae). This plant is regarded as phylogenetically close to such families as Lemnaceae and Araceae and, like the latter, has spikes of small unisexual flowers wrapped in fleshy bracts; unlike the arums, however, male and female flowers are mixed. The female flowers mature first and on the evening of the first day the inflorescence becomes invaded by night-flying beetles of the genus *Cyclocephala* (Scarabaeidae). What attracts them is uncertain, since no odour can be detected, but once within the inflorescence cavity they remain there overnight. By the afternoon of the second day, many can be observed in the act of copulation. At this stage the anthers dehisce, releasing masses of pollen into what is by now a moist and sticky environment among the enveloping bracts. Many of the beetles are apparently too engrossed in their activities to notice, on the evening of the second day, that the bracts have opened and exposed them, plastered in pollen, to the night air.

Undoubtedly some feeding goes on during this period, but the prime reason for the clandestine beetle gatherings within the *Cyclanthus* inflorescence is the shelter and protection it provides these rather slow and lumbering insects during their mating activities. Experimental exposure of the beetles by Beach during daylight hours confirmed that predation was intense, since they were rapidly consumed by both lizards and birds, such as toucans. On the other hand, experimental exclusion of beetles from the inflorescence led to its failure to produce seed. Here, then, is a mechanism by which a flower can offer its pollinator a most useful resource which involves very little energy expenditure on the part of the plant. □

Peter D. Moore is a lecturer in the Department of Plant Sciences, King's College, 68 Half Moon Lane, London SE24 9JF.

1. Faegri, K. & van der Pijl, L. *The Principles of Pollination Ecology* 3rd edn (Pergamon, London, 1979).
2. Proctor, M.C.F. & Yeo, P. *The Pollination of Flowers* (Collins, London, 1973).
3. Vogel, S. in *The Pollination of Flowers by Insects* (ed. Richards, A.J.) 89 (Academic, London, 1978).
4. Grant, V. *Evolution* 4, 179 (1950).
5. van der Pijl, L. *Evolution* 14, 403 (1960); 15, 44 (1961).
6. Grant, V. *Am. J. Bot.* 37, 294 (1950).
7. Rickson, F.R. *Am. J. Bot.* 66, 80 (1979).
8. Beach, J.H. *Am. J. Bot.* 69, 1074 (1982).

Oncogenes

Transcription of c-myc

from Peter Newmark

THREE new reports on the apparent consequences for the cellular oncogene, *c-myc*, of being translocated from its normal chromosome to one that carries the immunoglobulin constant-region locus in mouse plasmacytoma and in Burkitt's lymphoma (see this column, 13 Jan.) have blurred the original picture painted by Shen-Ong *et al.* (*Cell* 3, 443; 1982).

Shen-Ong *et al.* found no evidence of increased transcription of *c-myc* in any plasmacytoma but did detect a new shorter *c-myc* transcript. Now Marcu *et al.* (*Proc. natn. Acad. Sci. U.S.A.* 80, 519; 1983) claim that mouse plasmacytomas usually have a 10- to 20-fold increase in *c-myc* transcripts, a claim independently supported by J. Mushinski, S. Bauer, M. Potter and E. Reddy in a paper in press with the same journal. One possible explanation for the discrepancy lies in the different controls used by the three different groups; Mushinski *et al.* specifically raise the question of the most appropriate control in wondering whether elevated *c-myc* transcription in plasmacytomas might reflect the arrest of the cells at a developmental stage in which *c-myc* is abundantly transcribed rather than being a consequence of translocation of the gene.

Where all three groups are in better agreement is in detecting shorter than normal *c-myc* transcripts in those plasmacytomas where there is evidence of rearrangement of the *c-myc* gene in addition to its translocation. However, Mushinski *et al.* also find a large quantity of a shortened transcript in two plasmacytomas in which there is no evidence of *c-myc* rearrangement; and they note that shorter transcripts and/or rearrangement only occurred in plasmacytomas maintained in the laboratory for several years.

Parallel studies on Burkitt's lymphoma cell lines are included in the paper of Marcu *et al.* (op. cit.) and extended by J. Erikson, A. ar-Rushdi, H. Drwinga, P. Nowell and C. Croce in a paper soon to appear in *Proc. natn. Acad. Sci. U.S.A.* They present evidence of increased transcription of *c-myc* (5- to 10-fold), regardless of whether the gene shows signs of rearrangement.

Turning to the *c-ras* oncogene family, K. Shimizu *et al.* have now isolated, by molecular cloning, that gene from a human neuroblastoma cell line which is capable of transforming NIH 3T3 cells (*Proc. natn. Acad. Sci. U.S.A.* 80, 383; 1983). It is not, they report, grossly rearranged from the same gene in normal cells and though different from other *c-ras* oncogenes, it is a member of the family. □

Peter Newmark is Deputy Editor of Nature.

The auditory system

Binaural maps in the brain

from David R. Moore

A LONG search for the representation of a second sensory dimension in the central auditory pathway of the brain has, it seems, finally borne fruit. Several recent papers¹⁻³ have indicated that the primary auditory cortex has, in addition to its classic representation of sound frequency, an orthogonal banding of neurones with similar binaural response properties. John Middlebrooks and his colleagues at the University of California, San Francisco have now presented evidence⁴ that binaural bands may be a basic unit of organization throughout the central auditory pathway. By injecting different anatomical tracing compounds into one or more binaural bands in the cortex they showed that the sources of projections to these bands are themselves banded within the thalamus. These findings may turn out to be at least as important a step in understanding the organization of the auditory system as the discovery⁵ of cortical ocular dominance and orientation columns was in the visual system.

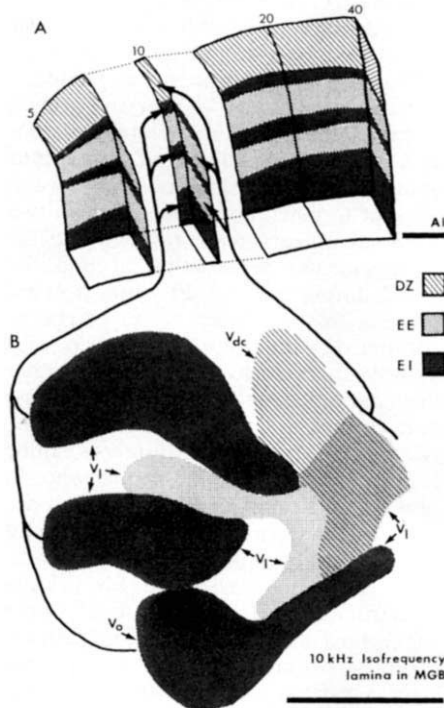
The sense of hearing has long been considered enigmatic among neuroscientists, as the fundamental mode of sound representation in the ear is unidimensional. In contrast to the visual and somatosensory (touch) receptors, which primarily code spatial attributes of stimuli, the auditory receptor sheet is organized like a frequency analyser. Sounds of high frequency produce peak activity of elements at one end of the organ of Corti — the sensory epithelium of the auditory system — and low-frequency sounds produce peak activity at the other end. This 'tonotopic' organization is preserved in all levels of the central auditory pathway. In the primary auditory cortex (AI) of the cat, for example, neurones at the caudal end of the field are most sensitive to low-frequency tones and those at the rostral end are sensitive to high-frequency tones⁶.

One consequence of the projection of an essentially one-dimensional structure (the organ of Corti) onto three-dimensional structures (the brain nuclei) is that the representation of frequency at higher levels is organized into two-dimensional sheets of neurones or 'isofrequency laminae'. Each lamina contains neurones most sensitive to a very narrow range of frequencies. In cat AI (see the figure), the isofrequency laminae form long narrow strips extending in a dorsoventral direction across the entire width of AI. In the principal thalamic source of projections to AI, the ventral nucleus of the medial geniculate body (MGv), and at lower levels of the auditory pathway, isofrequency laminae are arranged as sheets of neurones that approximately follow the surface contours

of each nucleus.

Over 30 years ago Tunturi⁷ noted that a frequency analyser can accomplish its function on a single element, and does not require a series of elements as each isofrequency lamina appears to be. It has thus long been believed that another dimension of sound was represented in the laminae. Only quite recently¹, however, has the representation of a second dimension in AI been demonstrated.

There are several reasons for this. In the 1960s and early 1970s, it was believed that AI was not strictly tonotopically organized. Although that belief is now largely seen to have arisen from the overlay of maps from several animals with different cortical sulcal patterns⁶, the observation⁸ still stands that, in unanaesthetized cats, neurones sampled from individual microelectrode penetrations oriented normally to the cortical surface may have a wide range of frequency selectivity. Anaesthetic state also seems to be a crucial factor in the demonstration of binaural bands. If neurones from superficial cortical layers (areas that are often inactive under deep



Binaural bands in the auditory cortex (AI) and thalamus (MGB) of the brain. The cortical representation of binaural responses (EE, EI) runs orthogonal to the representation of frequency (5–40 kHz isofrequency laminae). Neurones in discrete regions of the 10-kHz isofrequency lamina in the MGB (B) send their axons to separate binaural bands in AI (A). DZ is the dorsal zone of AI. V₁, V₂ and V₃ are subdivisions of the thalamus. (From ref. 4.)

barbiturate anaesthesia) are included in the analysis there is some doubt⁹ that the binaural interaction pattern is constant for neurones in a single microelectrode penetration through the depth of the cortex, let alone from one cortical surface position to another. A further difficulty arises from the definition of the interaction bands themselves. Middlebrooks' binaural bands consist of neurones displaying one of two types of response to binaural stimulation. Excitatory-excitatory (EE) neurones were excited monaurally by either ear, or had a binaural response that was larger than the response to monaural stimulation of the ear contralateral to the recording electrode. Excitatory-inhibitory (EI) neurones had a weaker response to binaural stimulation than to contralateral stimulation alone. Other researchers have used slightly different definitions of EE and EI neurones. Everyone agrees that a small proportion of AI neurones are only influenced by stimulation of one ear. It is also widely believed that many central auditory neurones receive a mixed excitatory-inhibitory input from both ears.

Despite these problems it now seems clear that it is possible to divide up binaural response properties in AI and that EE and EI neurones are arranged in bands orthogonal to the cortical isofrequency laminae.

In Middlebrooks' studies, each binaural band was defined with remarkable precision (see the figure). EE and EI bands seem most easily distinguishable at the rostral (high-frequency) end and along the ventral edge of AI. There is a clearly defined ventral pair of one EI (most ventral) and one EE band. Moving dorsally, there is a triplet of EI, EE and EI bands and, along the most dorsal edge of AI, a 'dorsal zone' of broadly tuned predominantly EE neurones.

Three types of retrograde anatomical tracer were injected into the bands to examine separately the sources of thalamic inputs (neurone cell bodies sending axons) to up to three AI loci in a single animal. Ventral EI band injections resulted in three discontinuous bands of labelled neurones in MGv, whereas ventral EE band injections resulted in a single continuous column of label in MGv. Injections into the middle EI and EE bands resulted in the same pattern, distribution and location of label in MGv as was seen with ventral injections, providing all injections were made in the same cortical isofrequency region. Dorsal-zone injections also labelled neurones in the EE zone of MGv. Within MGv, neurones located caudally generally project to cortical EI bands and rostral neurones project to EE bands.

These experiments have revealed a complex pattern of converging and diverging projections from MGv to AI. There is so far no physiological evidence to confirm the segregation of binaural response classes in MGv, but some segregation has been observed at the

inferior colliculus^{10,11}, the major source of projections to MGv. It thus seems likely that the binaural dimension will turn out to be represented across isofrequency laminae throughout the higher-order auditory nuclei. The final proof of this organization requires, as an initial step, a physiological study of MGv in which electrode penetrations are oriented along the thalamic EE and EI bands so elegantly demonstrated by Middlebrooks. □

David R. Moore is Departmental Demonstrator in Physiology, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT.

1. Imig, T.J. & Adrian, H.O. *Brain Res.* **138**, 241 (1977).
2. Imig, T.J. & Brugge, J.F. *J. comp. Neurol.* **182**, 637 (1978).
3. Middlebrooks, J.C., Dykes, R.W. & Merzenich, M.M. *Brain Res.* **181**, 31 (1980).
4. Middlebrooks, J.C. & Zook, J.M. *J. Neurosci.* (in the press).
5. Hubel, D.H. & Wiesel, T.N. *Proc. R. Soc. B198*, 1 (1977).
6. Merzenich, M.M., Knight, P.L. & Roth, G.L. *J. Neurophysiol.* **38**, 231 (1975).
7. Tunturi, A.R. *Am. J. Physiol.* **168**, 712 (1952).
8. Evans, E.F. in *Hearing Mechanisms in Vertebrates* (eds De Reuck, A.V.S. & Knight, J.) (Churchill, London, 1968).
9. Phillips, D.P. & Irvine, D.R.F. *Brain Res.* **161**, 342 (1979).
10. Roth, G.L., Aitkin, L.M., Andersen, R.A. & Merzenich, M.M. *J. comp. Neurol.* **182**, 661 (1978).
11. Semple, M.N. & Aitkin, L.M. *J. Neurophysiol.* **42**, 1626 (1979).

Stockholm meeting

Environmental priorities: international and supranational

from Norman Myers

IN late November 1982, the Royal Swedish Academy of Sciences convened a week-long gathering of eminent scientists to look at environmental priorities for the 1980s. The aim of the meeting was to see how far we have advanced, or lost ground, in our environmental efforts since the UN Conference on the Human Environment held in Stockholm 10 years earlier. The gathering brought together 35 leading scientists, from all over the world, representing more than one dozen disciplines. Hence the assembly was well able to assess the gigantic experiments that we are conducting with the planetary ecosystem — experiments whose outcome we can envisage in only vague terms at best.

At least 40 environmental issues had been targeted as worth consideration. The conference finally selected the following items for priority treatment on the grounds that they require increased efforts if we are to get on top of them. (1) Research priorities (not ranked in order of importance): tropical deforestation; loss of biological diversity; cryptic spread of mutant genes; impact of semi-periodic droughts and floods; energy needs; acid depositions; buildup of atmospheric carbon dioxide; impact of toxic emissions on ecosystems and humans; loss of cropland due to salinization; and urbanization. (2) Management priorities (again, not ranked in order of importance): toxic emissions; tropical deforestation; desertification; control of human-waste pathogens and their aquatic vectors; population growth, with special emphasis on urbanization; energy needs; acid depositions; fuelwood crisis; loss of species; and marine problems. When an item appears on one list but not on the other, this is because it is already receiving sufficient emphasis under the heading where it is not mentioned; for example, desertification does not merit further research than it is already receiving, but it deserves greatly increased management.

A number of environmental issues that do not appear in either of the two lists have also been recommended by the conference for enhanced attention from governments, international agencies and other 'decision makers'. They include groundwater stocks, the ozone layer, mining pollution, over-fishing of the oceans, environmentally sound technology, radioactive wastes and nutrient pollution of water. Unless we do a better job on these items of 'secondary importance', the adverse repercussions for us will, the conference determined, be profound and expensive.

These diverse problems are all grounded in two central factors — excessive growth of human numbers in developing nations and excessive growth of consumer appetites in advanced nations. Were we to attempt to determine which of these two phenomena bears greater responsibility for environmental degradation around the world during the past 10 years, it could prove a toss-up. The amount of energy consumed by the entire Third World, with its over-use of fuelwood stocks and other degradation of natural resources, is less than the amount of energy wasted by the rich world. Many similar instances can be cited, indicating that the people who do most harm to the Earth's ecosystems are half a billion super-rich people and one and a half billion ultra-poor people.

Of the items designated for priority treatment, only about half were highlighted at the Stockholm conference in 1972. An obvious newcomer is the energy issue in general; so is fuelwood as an energy problem in particular. Acid rain was hardly heard of 10 years ago; nor was tropical deforestation, desertification, or mass extinction of species. Little thought was given to increasing carbon dioxide in the global atmosphere, or to declining ozone in the stratosphere. Yet all these issues appear set to become increasingly conspicuous.

Whether directly or indirectly, these issues have the capacity to affect many, if

not most, nations at one and the same time — thus placing a premium on concerted endeavour by groups of nations, if not by the whole of humankind. The feasibility of a coordinated campaign at the international level is evident in the success of the clean-up plan for the Mediterranean and other regional seas — in contrast, most of the other broad-scale problems listed have stimulated little in the way of international response. In the case of desertification, the UN Conference on Desertification calculated that a remedial campaign would cost \$400 million; the amount so far supplied by the international community is \$10,000.

Environmental problems that have hitherto been perceived as intrinsically national in scope are starting to be regarded as international problems. For example, pollution is no longer so readily confined to national jurisdictions, as exemplified in the case of acid rain. Furthermore, international problems are increasingly perceived as supranational, if not global, problems. For example, mass extinction of species, while occurring in individual nation-states, will impoverish all humankind through loss of species' genetic resources and the contributions they could make to modern agriculture, medicine, industry and bio-engineering. Desertification and deforestation may well lead to global climatic disruptions, while depletion of the ozone layer will harm the guilty and the innocent alike. □

Norman Myers is a Consultant in Environment and Development, Upper Meadow, Old Road, Headington, Oxford OX3 9AP.



100 years ago

LIEUT. WISSMANN, the intrepid and successful German traveller, arrived at Cairo on January 1. His route from Loanda, by way of Nyangwe, on the Lualaba River, to Zanzibar, which measures about 3600 kilometres, led him for at least one-third of the distance through unexplored country. He has thus solved some of the enigmas of equatorial Africa. It is the southern half of the Congo basin through which Wissmann passed, and he found this to be most densely populated. This fact is remarkable, as it was entirely unexpected. Wissmann also passed through the land of a tribe of dwarf negroes. On the long and dangerous route from Lake Tanganyika to Zanzibar the traveller met with a most hospitable reception at the hands of the renowned brigand chief Mirambo, who supported him in every respect.

A member of the Paris "Ecole pratique d'acclimatation" has discovered a species of spider on the African coast, the firm and long web of which resembles yellow silk very closely, and is said to be almost as good as the product of real silkworms. The syndicate of the Lyons silk-merchants has closely investigated the matter, and the result is reported as highly favourable. There seems to be no difficulty in the way of acclimatizing the new silk-producer in France. From *Nature* 27, 348, 8 February 1883.

Royal Society meeting

Sputniks I and II—25 years ago

from F. Graham Smith

TWENTY-FIVE years ago, in October 1957, the launch of Sputnik I took radio scientists everywhere by surprise. To mark the anniversary, Sir Harrie Massey and the British National Committee for Space Research brought some of the British observers together in a nostalgic review meeting at the Royal Society on 3 December.

The most exciting and momentous story came from Sir Bernard Lovell, who recounted the few hectic days when the 250-ft radio telescope was rapidly adapted for radar observations of the rocket launcher¹, with such success that the financial crisis of the telescope was resolved very soon afterwards. The Cambridge radio astronomers worked on the satellites themselves, using interferometer and Doppler techniques to find the orbits of Sputnik I and Sputnik II. They were able to start with a simple re-tuning of a 38 MHz receiver to pick up the 40 MHz telemetry. All over the world there were similar responses to the bleep-bleep of Sputnik I, an event which was at the same time so easily predictable and yet so unexpected.

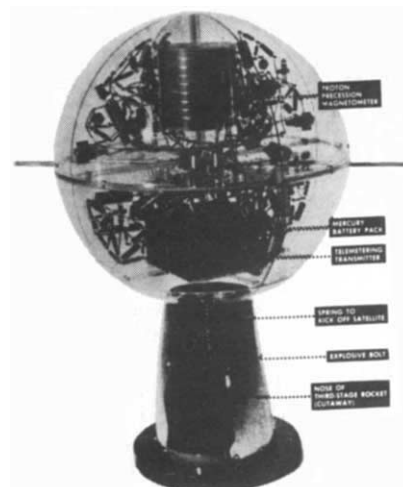
There was, of course, a deeper understanding in military circles. The general ignorance in the universities before the launch, followed by intense interest afterwards, could probably be documented through the sudden proliferation of examination questions on satellites and their orbits. In contrast, the Royal Aircraft Establishment had for several years studied the design and possible performance of a one-ton Earth Satellite reconnaissance vehicle, to be launched by a British launcher. Desmond King-Hele gave an account of his work with Doreen Walker (or Gilmore as she then was), which showed that the Royal Aircraft Establishment already had theories for the effect of air-drag and for the gravitational effect of the Earth's oblateness on satellite orbits.

There was, of course, a good appreciation by the space research groups of the potential usefulness of artificial satellites for atmospheric research and for

astronomy. In fact the chief protagonists were, at the time of launch, at a meeting in Washington DC, along with their colleagues from the US and the Soviet Union. There is a story that misreporting of this meeting in the Soviet Union, leading to a belief that the US was just on the point of launching a satellite, was responsible for the actual date of launch being advanced by some weeks. In the event, the announcement in Washington of the successful launch had a stunning effect. Doubtless the military in the US made their own observations, but these were not published. It was left to Cambridge to publish the first orbit², followed soon after by the Royal Aircraft Establishment³. Within four weeks, the radio techniques had been developed from scratch, and the observations had yielded useful values for the rate of change of orbital period, which could be related to air density.

King-Hele showed an interesting comparison between the two published sets of orbital parameters and a more accurate set derived in retrospect (see the table). The agreement is remarkably good, considering especially the lack of expertise in orbital theory at Cambridge. Here some help should be acknowledged: the then science reporter of the Guardian, one John Maddox, contributed a copy of an American work on orbits, which helped us understand the subtleties of precession round an oblate spheroid. The Royal Greenwich Observatory also helped, although their preference was to wait for the more precise data that could be expected from visual observations.

Both groups used the rate of change of period to obtain a value of air density. King-Hele showed at this meeting that the high value (2×10^{-10} of sea-level density at a height of 241 km) published by the Royal Aircraft Establishment required only a small correction (25 per cent) for an underestimate of the cross-sectional area of Sputnik I, and that it has a unique importance in atmospheric physics. By coincidence there was an exceptionally high value of solar activity at the time, and it now



Sputnik I

appears that the high value for air density, which is about 20 per cent higher than the standard COSPAR International Reference Atmosphere, was correctly determined.

Sputnik II was observed optically, enabling the Royal Aircraft Establishment to obtain a very much more accurate orbit by the use of kinetheodolites. This led to several remarkable discoveries concerning the rotation rate⁴ and density variations⁵ in the atmosphere, and the oblateness of the Earth⁶. The era of precise orbital analysis had begun.

Looking back on those few months, which ended with the spectacular fireworks display made by Sputnik II as it came down over the Caribbean Sea on 14 April 1958, one sees significant consequences for all the British protagonists. At Jodrell Bank there was a rapid switch from meteor radar to lunar radar, with its consequences in both communications and ionospheric research. The Royal Aircraft Establishment became the centre of the National Prediction Service and a world leader in the analysis of the gravitational field of the Earth. At Cambridge there was a rapid return to the more serious business of determining the structure of the Universe; there was, however, a notable publication soon afterwards, which proved to be the first note on the use of satellites for navigation through the Doppler effect⁷, and the seeds had been sown for low-frequency observations from satellites. For many others, the Sputniks will be remembered as heralds of a great age of space exploration, which in Britain centred on the very successful series of six Ariel satellites. □

Orbital parameters of Sputnik I

	Cambridge	Farnborough	In retrospect
Epoch	1957 October 15.0	1957 October 15.0*	1957 October 15.0
Nodal period	95 min 50.0 ± 0.3 s	95 min 49.6 s	95 min 49.6 s
decreasing at (s per day)	2.2 ± 0.1	2.3	2.3 ± 0.1
Eccentricity	0.053 ± 0.001	0.048 ± 0.002	0.050 ± 0.001
Perigee height (km)	197 ± 10	230 ± 13	224 ± 5
Perigee latitude	36°N ± 3°	41°N	47°N ± 2° (?)

*Royal Aircraft Establishment values converted from October 15.5. The parameters were originally determined in October 1957 by the Mullard Observatory, Cambridge and the Royal Aircraft Establishment, Farnborough; they were redetermined later.

F. Graham Smith is Director of the Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire SK11 9DL.

1. Staff of Jodrell Bank *Nature* **180**, 941 (1957); *Proc. R. Soc. A* **248**, 24 (1958).
2. Staff of Mullard Observatory *Nature* **180**, 879 (1957).
3. Staff of Royal Aircraft Establishment *Nature* **180**, 937 (1957).
4. Merson, R.H., King-Hele, D.G. & Plimmer, R.N.A. *Nature* **183**, 239 (1959).
5. King-Hele, D.G. & Leslie, D.C.M. *Nature* **181**, 1761 (1958).
6. Merson, R.H. & King-Hele, D.G. *Nature* **182**, 640 (1958).
7. Smith, F.G. *J. Inst. Navigat.* **13**, 109 (1960).

Environmental pollution

Reproductive success of eagles and organochlorine insecticides

from Robert M. May

A VARIETY of studies have suggested that the insecticide DDT and associated compounds into which it is metabolized are responsible for diminished reproductive success in many species of birds. These problems tend to be most pronounced for birds of prey, which sit at the top of food chains in which the toxins are concentrated from link to link. The metabolite dichlorodiphenyl-dichloroethylene (DDE) probably poses the greatest physiological threat to birds of prey, being the most persistent contaminate both in the environment and in the bodies of birds. Indeed, so stable in the environment is DDE that Beyer and Gish (*J. appl. Ecol.* 17, 295; 1980) could not detect any significant rate of decay over an 11-year study.

In the US, the Environmental Protection Agency banned further use of DDT at the end of 1972. There have subsequently been reports of increased reproductive success (and decreased DDE contamination) in several species of birds of prey, notably in the ospreys in Connecticut and on Long Island (P. Spitzer *et al. Science* 202, 333; 1978).

It is perhaps fitting that the most detailed study yet published is for the bald eagle, *Haliaeetus leucocephalus*, the national bird whose symbolic representation is so ubiquitous in the US, while the species itself is on the endangered list. The study by Grier (*Science* 218, 1232; 1982) is actually for a population in northwestern Ontario; the birds nest in a part of Canada that has suffered little exposure to DDT, but they spend the winter in the US where they consume prey contaminated with DDT.

Grier presents data, reaching back to 1966, on the average number of young produced annually in each 'breeding area'. The breeding areas are akin to nesting territories: an area 'may contain several alternative nests, but only one nest is used for raising young during any year'. The production of young is defined as the number raised to the late nestling stage, which is the last stage at which they can reliably be counted. Grier shows that reproduction declined steadily throughout the late 1960s and the early 1970s, from 1.26 young per breeding area in 1966 to a low of 0.46 in 1974. The subsequent history is one of steady increase, to 1.12 in 1981. The patterns can be confirmed by several kinds of statistical analysis. For example, if the 16 years of the study are divided into two segments, corresponding to years before the ban and to years after, both segments can be well fit by a linear regression line (with slopes of -0.07 and

$+0.07$ young per breeding area per year respectively); the difference between these slopes is highly significant.

Associated with this increasing reproductive success following the banning of DDT is a marked decline in the mean concentration of DDE residues found in addled eggs. Specifically, the DDE concentration in eggs averaged around 100 parts per million (by weight) before 1972 and dropped very significantly to an average of around 30 p.p.m. after 1975.

As Grier emphasizes, these results raise new questions every bit as interesting as the questions they answer. Even if DDE is diffusing comparatively rapidly out of the bald eagles' food web — by settling into sediments, for instance — we would expect the toxin to be flushed slowly from the bodies of the birds themselves, as it is 'thought to be eliminated from female birds only through fat deposited in eggs'. In black ducks, *Anas rubripes*, the shell

thinning and general reproductive impairment caused by DDE has been shown to persist long after the birds resume an uncontaminated diet (J. Longcore & R. Stendell *Arch. environ. Contam. Toxicol.* 6, 293; 1977). The simplest way to reconcile such persistent contamination of individuals with the pattern of population recovery seen above is to assume that young uncontaminated female eagles are replacing older contaminated breeding females significantly more rapidly than is thought to be the case for such populations. Of course, it could be that the conventional wisdom is at fault, and turnover always has been relatively rapid. But it could equally be that such faster turnover in the breeding population is associated with decreasing survival rates among adults.

In short, a full understanding of the dynamical behaviour of a population requires knowledge about both birth rates and survivorship. Grier has shed important light on fertility patterns among his bald eagles, but we also need information about age-specific survival rates. It could be that these bald eagle populations have troubles beyond those associated with DDT. □

Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

Earth science

The origin of ribbon radiolarites

from Peter J. Smith

RIBBON radiolarites are rocks that derive their name from their characteristically repetitive bedding — they comprise layers of radiolarian chert (grossly recrystallized radiolaria in a microcrystalline quartz matrix) centimetres to tens of centimetres thick, usually alternating with layers of argillite (clay) generally no more than a few millimetres thick. Complete sequences are typically tens to hundreds of metres thick. Very occasionally the clay layers are comparable in thickness with the chert layers; but more often the argillite is not there at all, only a parting separating one quartzose layer from the next. Where the chert–argillite couplet is present, however, the two elements sometimes form a single graded unit and sometimes appear to be independent.

The origin of the chert–argillite pairing is a puzzle, but it is not the only one associated with ribbon radiolarites. For example, no rock remotely resembling ribbon radiolarite has ever been found in cores taken from present-day ocean basins. No thick, stratigraphically continuous chert sequences have been recorded there at all, and there is no sign of chert–argillite couplets. (Cherts do, of course, exist in the ocean basins but they are lenticular, not ribboned.)

As for the time dimension, ribbon

radiolarites are characteristic of the Mesozoic and parts of the Palaeozoic, radiolaria having reached their peak in the Jurassic. The siliceous record of the Cenozoic, by contrast, is dominated not by radiolarian cherts but by diatomites, diatoms having originated during the Jurassic but having been particularly well developed during the Tertiary (Miocene).

The other conspicuous characteristic of ribbon radiolarites is that they are apparently restricted to orogenic belts, most commonly occurring as parts of thrust sheets, as olistoliths (exotic blocks), or in *mélanges*. They exist, for example, throughout the Alpine–Mediterranean (Tethyan) region, although on a small scale they are highly diachronous — they formed in different localities over slightly different periods. Nor do they all share quite the same associations. In the western Tethys they often lie on Jurassic pelagic limestones which pass downwards through other sedimentary rocks to continental basement; the complete sequences can be regarded as foundered Atlantic-type continental margins. In some Tethyan areas, on the other hand, ribbon radiolarities lie above ophiolite sequences, as they also do in Cyprus and the Oman; the sequences there lie above material of oceanic origin. In parts of California they also lie above

ophiolites as well as in the Franciscan, a *mélange* of mafic and ultramafic rocks, blueschists and diverse sediments.

Any model for the deposition and diagenesis of ribbon radiolarites must clearly take all these characteristics into account and, as Jenkyns and Winterer demonstrate (*Earth planet. Sci. Lett.* **60**, 351; 1982), any attempt to do less can easily mislead. For instance, the fact that all ribbon radiolarites seem to have been involved in orogenic processes, combined with the fact that mature diatomites that have been through tectonic upheaval also display ribboning, might be taken to suggest that ribbon radiolarites were generated during orogenesis by the action of temperature and pressure on a more or less homogeneous mixture of clays and radiolarian-derived silica. But that cannot have been the case, for the examination of slump folds clearly shows that the chert–argillite pairing was present when the sediment was soft enough to be deformed. The pairing must either have been primary or the result of very early diagenetic segregation into silica-rich and silica-poor units, or perhaps a combination of the two.

If it was primary, there are several obvious possibilities. One is that more or less continuous radiolarian deposition was interrupted from time to time by the injection of redeposited clay, an idea originated by Birkenmayer and Gasiorowski (*Bull. Acad. Polish Sci., Sér. Géol. Géogr.* **9**, 171; 1961). However, Jenkyns and Winterer conclude that, on balance, the more likely variable was the radiolarian productivity, presumably influenced by climate. In this case, radiolaria would have been deposited or redeposited episodically at rates much higher than that of the continuous background of clay deposition, producing a primary chert–argillite alternation that was perhaps accentuated by later diagenesis.

But the radiolarian deposition rates, though high, must also have been variable, for it is evident that the total rates of ribbon radiolarite accumulation were very different at different sites. At the same time, however, the thicknesses of the chert and argillite layers are comparable from site to site; there is no suggestion, as with some other well known sedimentary couplets, that thicker sequences incorporate thicker layers. This particular combination of circumstances could only arise, as Jenkyns and Winterer argue in their central thesis, if deposition had occurred in oceanic basins of 'relatively modest' dimensions.

Supporting evidence for this view comes from a number of different fields. Geologically, for example, ribbon radiolarites are frequently associated with ophiolite sequences; and ophiolites are widely believed to be ancient fragments of ocean floor generated in small newly forming ocean basins or arc-related marginal basins — that is, in near-continental environments. The near-continental theme also

arises in connection with the geochemistry, in particular that of the rare earths. Siliceous sediments laid down in the open oceans have rare earth abundance signatures quite different from those of marginal sediments influenced by continental contamination. Thus, for instance, open ocean cherts generally have large negative caesium anomalies, whereas near-continental ones have small or non-existent caesium anomalies — as do many (but not all) ribbon radiolarities. Finally, there is palaeontological support. The rocks overlying ribbon radiolarites frequently comprise micrites containing the coccolithophorid *Nannoconus*, which is believed to be a near-continental, as opposed to an open-ocean, form.

The conclusion that Jenkyns and Winterer come to from this and a considerable amount of other evidence is that most, if not all, Mesozoic ribbon radiolarites originated in small ocean basins and on the

deeper of continental margins in the western Tethys. Formation in the global oceans would be consistent neither with the great variations in the thicknesses of ribbon radiolarite sequences nor with the differences in the precise times of their formation. Moreover, as detailed analysis suggests, only in small ocean basins and comparable features would the calcite compensation depth during the Jurassic have been elevated enough to allow siliceous deposition to take place. The corollary of this conclusion, of course, is that whereas the examination of ribbon radiolarites may provide information about local palaeoceanographic, palaeotectonic and sedimentary environments, it can lead to little increase in knowledge of conditions in the Mesozoic World Ocean. □

Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes MK7 6AA, Buckinghamshire, and editor of *Open Earth*.

Laser spectroscopy

Multiphoton techniques expand combustion diagnostic potential

from Kermit C. Smyth

WITH the advent of convenient and readily tunable laser sources, particularly in the visible and UV regions of the spectrum, optical techniques have been increasingly used in flame environments for species identification and concentration measurements. Recently, multiphoton ionization experiments carried out by J.E.M. Goldsmith have provided a high-sensitivity technique for the observation of hydrogen and oxygen atoms^{1,2}. Goldsmith's results represent the first direct optical detection of hydrogen atoms and the most sensitive detection so far of oxygen atoms in a flame.

In the past the most popular optical method for detecting minor species, such as the radicals which play an important part in flame chemistry, has been laser-induced fluorescence. The technique has the advantages inherent in an optical measurement: it is non-intrusive, species specific, highly sensitive, and capable of excellent spatial and temporal resolution. Over 20 diatomic and triatomic molecules have been detected and studied in flames using laser-induced fluorescence.

None of the interesting atomic species (such as H, O, C and N) has, however, been observed using single-photon excitation followed by emission. All these atoms exhibit a very large energy gap — approximately 10 eV — between their ground state and their first optically allowed excited electronic state, necessitating photon energies in the vacuum UV region. Multiphoton ionization spectroscopy can successfully circumvent the problems of absorption by window material and atmosphere that

occurs with vacuum UV radiation by using two or more photons of longer wavelength. In addition, the multiphoton experiments retain most of the key advantages of the older optical techniques.

The importance of hydrogen and oxygen atoms in combustion chemistry is very great but most techniques devised for their detection³ are indirect and involve spectroscopic or mass spectrometric observation of some species other than the atom of interest; probe sampling is often required. There is a strong need for a direct optical method for measurement of the absolute concentration of atomic species in a variety of combustion conditions.

In Goldsmith's experiments, two near-UV photons were used to excite a resonant transition, and then absorption of a third photon caused ionization of either the hydrogen or oxygen atoms. The approach is very similar to the multiphoton ionization technique used to monitor hydrogen atoms in discharges and flow cells⁴⁻⁶. The photoelectrons and the photoions produced by the absorption of three photons can then be readily detected by using an electrode in electrical contact with the flame. The electrode may be located many millimetres away from the focal volume of the laser beams, thus avoiding severe flow perturbation.

The multiphoton ionization studies can be carried out using high-power lasers with pulse durations of under 10 ns, so affording rapid data acquisition. When two distinct wavelengths are used, as in the hydrogen atom work¹, a crossed-beam geometry can also provide excellent spatial

resolution. Most important, multiphoton ionization is highly sensitive, with detection limits estimated to be at the parts per million (p.p.m.) level^{1,2}. In contrast, Raman studies on oxygen atom detection in flames have a detection limit of one per cent^{7,8}.

In circumstances where the detection of ionization events by an electrode is inconvenient or causes unacceptable perturbations, multiphoton excitation followed by the observation of fluorescence may prove to be most useful. Studies have been reported on hydrogen⁶, carbon⁹, nitrogen^{10,11}, oxygen⁹⁻¹¹ and chlorine atoms¹² — all in low-pressure environments. Bischel, Perry and Crosley¹⁰ estimated that sensitivities in the p.p.m. range are possible for oxygen and nitrogen atom detection in a flame using fluorescence induced by two-photon absorption. Aldén *et al.*¹³, the first to apply the technique to flame conditions, successfully detected oxygen atoms. They estimated a detection limit of 0.1 per cent. Any of the atoms listed above that can be detected by fluorescence following two-photon absorption are also obvious candidates for study by three-photon ionization.

The multiphoton ionization techniques also hold great promise for polyatomic molecules. Many species which fluoresce at low pressure do not emit readily in a flame at one atmosphere, where electronic quenching is rapid. For example, NO and PO are difficult to detect by fluorescence, yet both can be easily observed using multiphoton ionization^{14,15} even when four photons are required¹⁶. For these diatomic molecules two-photon ionization gives sensitivities below 1 p.p.m., at least one hundred times more sensitive than fluorescence detection. It will be interesting to see whether multiphoton ionization methods can be extended to important radicals such as CH₃· and NH₂·, which have not been observed by fluorescence in flames. Considerable multiphoton ionization work on methyl radicals has been reported in a low-

pressure pyrolysis cell by DiGiuseppe, Hudgens and Lin¹⁷.

What does the future hold for multiphoton detection of flame species? The high sensitivities demonstrated in premixed flames by the multiphoton ionization methods will certainly encourage their application to the study of both atomic and polyatomic species, particularly those not accessible by fluorescence techniques. None of the multiphoton optical approaches has, however, yielded quantitative concentration data. This is certainly the most important attribute of a useful diagnostic

technique. Quantitative results will be especially difficult to obtain for polyatomic species, since the effects of collisional quenching and rapid energy transfer must be understood in some detail. Finally, diffusion flames await study by all the multiphoton techniques. Such environments pose additional challenges, such as low flow velocities and substantial soot concentrations in hydrocarbon flames. Much exciting study lies ahead. □

Kermit C. Smyth is in the National Bureau of Standards, Washington DC 20234.

Eukaryotic gene expression

Very short repeats and coordinate induction of genes

from Eric H. Davidson, Howard T. Jacobs and Roy J. Britten

THE proposition that a set of unlinked genes could be coordinately induced by means of *cis* interactions with a common regulatory molecule retains an attractive simplicity. The idea requires that some form of homologous sequence exists in the vicinity of genes that are functionally related and are induced in concert. As primary sequence data for sets of such genes have become available, several interesting homologies have indeed turned up. Though the shared sequence elements are quite short, the statistical probability of their chance occurrence in similar positions with respect to each of the several coordinately induced genes is extremely low. Furthermore, in two such cases, direct evidence has been obtained by DNA transformation that the short repeated sequence elements shared among the 5'-flanking regions of the inducible genes are required for regulation.

In the table (p.470), we have collected data from five different sets of genes. Each set consists of several structural genes that are transcriptionally activated in response to the same specific change in environmental circumstance, or by a common effector molecule. Though the homologies are always imperfect, in all five examples a similar sequence element occurs in the 5'-flanking region of each gene of the set.

The most convincing case in the table concerns four yeast genes for amino acid synthesis that are activated when the organism is starved of amino acids. The evidence just reported by Donahue *et al.*¹ in the latest issue of *Cell* shows that a single copy of the repeated sequence in the 5' region of the yeast *his4* gene suffices to bring the gene under inductive regulation, provided that the adjacent promoter sequences are present as well. In transformation experiments, deletion mutants of the gene that lack the repeated sequence element cannot be induced. A similar result was obtained by Struhl², who

identified the analogous regulatory sequence in the 5' region of the *his3* gene. Rare revertants of the *his4* deletion mutants occur, and on examination of these, Donahue *et al.* found that the six-nucleotide core of the repeated consensus sequence shown in the table, 5' TGACTC, had been recreated in each case by a single base pair change occurring in an appropriate location in the deletion mutants. The return of *his4* inducibility thus depends specifically on the restoration of the sequence element that is shared with other genes responding to amino acid starvation.

Transformation experiments also directly support the functional significance of the short repeat shared among the *Drosophila* heat-shock genes. Pelham³ used a transient expression vector introduced into cultured mammalian cells, in which the *Drosophila hsp70* gene responds transcriptionally to heat shock. Deletions that affect the shared repeat sequence obliterate the heat response in this system. Furthermore, Pelham showed that ligation of the *hsp70* 5'-flanking sequences to the herpes thymidine kinase gene causes the latter to become inducible by heat, and this effect is not seen if the *hsp70* DNA included lacks the shared repeat sequence element. The fact that the regulation operates in a phylogenetically distant organism, and in a different temperature range from that in *Drosophila*, shows that the heat response is a property of the interaction of the DNA with a *trans*-acting factor. The key features of the recognition sequence must have been very highly conserved in evolution.

There is no direct evidence for the regulatory significance of the shared repeat sequences in the other three cases listed in the table. The circumstantial argument that leads to their inclusion is the improbability of their chance occurrence: an approximate calculation (see footnote** of the table) showed that the probability of

1. Goldsmith, J.E.M. *Optics Lett.* 7, 437 (1982).
2. Goldsmith, J.E.M. *J. chem. Phys.* (in the press).
3. Hastie, J.W. *High Temperature Vapors*, 263-268 and refs therein (Academic, New York, 1975).
4. Bjorklund, G.C., Ausschnitt, C.P., Freeman, R.R. & Storz, R.H. *Appl. Phys. Lett.* 33, 54 (1978).
5. Ausschnitt, C.P., Bjorklund, G.C. & Freeman, R.R. *Appl. Phys. Lett.* 33, 851 (1978).
6. Bokor, J., Freeman, R.R., White, J.C. & Storz, R.H. *Phys. Rev. A24*, 612 (1981).
7. Dasch, C.J. & Bechtel, J.H. *Optics Lett.* 6, 36 (1981).
8. Teets, R.E. & Bechtel, J.H. *Optics Lett.* 6, 458 (1981).
9. Müller, C.H. III, Eames, D.R. & Burrell, K.H. *Bull. Am. phys. Soc.* 26, 1031 (1981).
10. Bischel, W.K., Perry, B.E. & Crosley, D.R. *Chem. Phys. Lett.* 82, 85 (1981).
11. Bischel, W.K., Perry, B.E. & Crosley, D.R. *Appl. Optics* 21, 1419 (1982).
12. Heaven, M., Miller, T.A., Freeman, R.R., White, J.C. & Bokor, J. *Chem. Phys. Lett.* 86, 458 (1982).
13. Aldén, M., Edner, H., Grafström, P. & Svanberg, S. *Optics Commun.* 42, 244 (1982).
14. Mallard, W.G., Miller, J.H. & Smyth, K.C. *J. chem. Phys.* 76, 3483 (1982).
15. Smyth, K.C. & Mallard, W.G. *J. chem. Phys.* 77, 1779 (1982).
16. Rockney, B.H., Cool, T.A. & Grant, E.R. *Chem. Phys. Lett.* 87, 141 (1982).
17. DiGiuseppe, T.G., Hudgens, J.W. & Lin, M.C. *Chem. Phys. Lett.* 82, 267 (1981); *J. Phys. Chem.* 86, 36 (1982); *J. chem. Phys.* 76, 3337 (1982).

chance occurrence anywhere in the same 100-nucleotide region flanking the transcriptional start site of all three glucocorticoid-regulated genes of the mismatched repeat sequences reported by Cochet *et al.*⁴ is less than 10^{-8} . The chance likelihood of occurrence in the same 12 nucleotides of the 5'-flanking sequence of the genes for the three steroid-induced chicken egg-white proteins is less than 10^{-5} . No attempt was made to calculate the probability of chance occurrence for the chorion gene repeats, since the structural genes themselves are ancestrally related and have evidently evolved as a series of duplications. Furthermore, they have retained a clustered organization that might facilitate sequence conservation. Except for the shared repeats, however, most (~60 per cent) of the 5' sequence between -160 and the TATAA boxes at -25 to -30 is nonhomologous within these chorion gene pairs.

An important feature of the shared repetitive sequences is their very short length. Of the consensus sequences listed in the table, the longest is 24 nucleotides (the rodent and human genes responding to glucocorticoids) and the shortest are 9 nucleotides (the yeast amino acid synthesis genes and the chicken egg-white protein genes). In the case of the yeast genes, the repetitive sequence occurs at multiple sites in the 5'-flanking region of each of the four genes compared but, as noted, only one occurrence suffices for inductive control¹. This feature may occur frequently. Sequences with considerable resemblance to the canonical repeats are found at additional positions in the 5'-flanking regions of at least some of the *Drosophila* heat-shock genes and of the chick lysozyme gene. In the cases of the yeast amino acid synthesis genes and the heat-shock protein genes of *Drosophila*, the respective authors^{1,3} have pointed out a further interesting feature — that the repeated element forms, or belongs to, an imperfect palindromic sequence. Palindromic sequences have been noted in the putative control regions of a number of other eukaryotic genes as well⁵⁻⁷ and they may be of functional significance. In the other examples listed in the table, however, obvious palindromic sequences are lacking.

Comparison of the six consensus sequences in the table demonstrates that they are all different. The silk moth chorion genes illustrate this diversity nicely. The consensus sequence for the two late genes is not homologous with that for the two middle genes included in the comparison. Different regulatory sequences would be required if they were in fact involved in induction, since these gene sets must respond to different regulatory effectors in order to begin functioning at different times within the same follicle cells. The fact that each of the shared sequence elements in the table is unique to its own set of genes argues that these

sequences are not simply basic eukaryotic promoter elements, in contrast, for example, to the TATAA box. Specific support for this view comes from the yeast amino acid synthesis genes. Hinnebusch and Fink⁸ found that the essential 5' TGACTC core sequence is absent from the 5'-flanking region of eight other sequenced yeast genes, none of which is regulated specifically by amino acid starvation. Furthermore, though it destroys inducibility, deletion of the shared sequence element from the *his4* gene does not affect the basal level of *his4* transcript. Donahue *et al.*¹ thus argue that the core sequence is a component required specifically for induction, rather than a fundamental promoter element. The same argument for a specifically inductive role of the shared sequence element can be made in the case of the *Drosophila* heat-shock genes. Though deletions of the *hsp70* shared sequence element eliminate heat inducibility, they do not prevent constitutive transcription, including a very high level of transcription when the transforming construct includes a SV40 72-nucleotide enhancer sequence inserted 5' to the gene.

The idea that repetitive *cis* recognition

1. Donahue, T.F., Davis, R.S., Lucchini, G. & Fink, G.R. *Cell* **32**, 89 (1983).
2. Struhl, K. *Nature* **300**, 284 (1982).
3. Pelham, H.R.B. *Cell* **30**, 517 (1982).
4. Cochet, M., Chang, A.C.Y. & Cohen, S.N. *Nature* **297**, 335 (1982).
5. Grosschedl, R. & Birnstiel, M.L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7102 (1980).
6. Groudine, M. & Weintraub, H. *Cell* **30**, 131 (1982).
7. McKnight, S.L. & Kingbury, R. *Science* **217**, 316 (1982).
8. Hinnebusch, A.G. & Fink, G.R. *J. biol. Chem.* (submitted).
9. Britten, R.J. & Davidson, E.H. *Science* **165**, 349 (1969).
10. Davidson, E.H. & Posakony, J.W. *Nature* **297**, 633 (1982).
11. Davidson, E.H., Hough, B.R., Klein, W.H. & Britten, R.J. *Cell* **4**, 217 (1975).
12. Cochet, M. *et al.* *Nature* **282**, 567 (1979).
13. Bell, G.I., Pictet, R. & Rutter, W.J. *Nucleic Acids Res.* **8**, 4091 (1980).
14. Proudfoot, N.J., Shander, M.H.M., Manley, J.L., Gefter, M.L. & Maniatis, T. *Science* **209**, 1329 (1980).
15. Shen, C.-K. J. & Maniatis, T. *Cell* **19**, 379 (1980).
16. Page, G.S., Smith, S. & Goodman, H.M. *Nucleic Acids Res.* **9**, 2087 (1981).
17. Pearson, W.R., Mukai, T. & Morrow, J.F. *J. biol. Chem.* **256**, 4033 (1981).
18. Ryffel, G.U., Meullener, D.B., Wyler, T., Wahli, W. & Weber, R. *Nature* **291**, 429 (1981).
19. Young, P.R., Scott, R.W., Hamer, D.H. & Tilghman, S.M. *Nucleic Acids Res.* **10**, 3099 (1982).
20. Schibler, U. *et al.* *J. molec. Biol.* **155**, 247 (1982).
21. Britten, R.J. & Davidson, E.H. *Q. Rev. Biol.* **46**, 111 (1971).
22. Wilson, A.C., Sarich, V.M. & Maxson, L.R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3028 (1974).
23. Davidson, E.H. in *Evolution and Development* (ed. Bonner, J.T.) 65 (Springer, Berlin, 1982).
24. Donahue, T.F., Farabaugh, P.J. & Fink, G.R. *Gene* **18**, 47 (1982).
25. Struhl, K. & Davis, R.W. *J. molec. Biol.* **152**, 553 (1981).
26. Zalkin, H. & Yanofsky, C. *J. biol. Chem.* **257**, 1491 (1982).
27. Ingolia, T.D. & Craig, E.A. *Nucleic Acids Res.* **9**, 1627 (1981).
28. Holmgren, R., Corces, V., Morimoto, R., Blackman, R. & Meselson, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3775 (1981).
29. Karch, F., Török, I. & Tissières, A. *J. molec. Biol.* **148**, 219 (1981).
30. Jones, C.W. & Kafatos, F.C. *Cell* **22**, 855 (1980).
31. Bartia, A., Richards, R.I., Baxter, J.D. & Shine, J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4867 (1981).
32. Donehower, L.A., Huang, A.L. & Hager, G.L. *J. Virol.* **37**, 226 (1981).
33. Groz, M. *et al.* *Cell* **25**, 743 (1981).
34. Dykes, G., Bambara, R., Mariani, K. & Wu, R. *Nucleic Acids Res.* **2**, 327 (1975).

sequences might provide the structural basis for the coordinate induction of unlinked structural genes was elaborated by Britten and Davidson⁹. The proposed regulatory elements were designated 'receptor' sequences. In addition to the receptor sequences and the structural genes themselves, three additional components were logically required for this hypothetical form of coordinate regulation. These were the *trans*-'activators' that specifically bind at the receptor sequences; the genes that produce such activators, termed 'integrator' genes (in that the role of each is to functionally integrate the activities of a set of disparate structural genes); and 'sensor' sequences, conceived as genomic sites that can induce activator synthesis in response to change in external circumstances (for example, amino acid starvation, heat, or the advent of an exogenous steroid). The observations summarized in the table provide some tangible evidence for the first component of this regulatory network, if 5' repeat sequences shared among coordinately induced genes such as those in the table indeed function as envisioned for receptor sequences. Questions are then raised of the nature of the activator molecules that interact with them, the mechanism of that interaction and the role of the sensor sequences that enable them to perform their function.

There have been extensive efforts to detect repetitive sequences in the vicinity of structural genes by DNA renaturation methods. As generally applied, however, the sensitivity of these methods does not extend to sequence elements as short as the consensus sequences listed in the table, and homologies such as these could not have been detected. Instead renaturation analyses have revealed an enormous menagerie of longer repetitive sequences, ranging from a few hundred to several thousand nucleotides in length, which are now established as a prominent feature of both animal and plant genomes. Repetitive sequences of this nature are interspersed throughout the genomic DNA, including, at least in some mammalian and echinoderm systems, a large fraction of nuclear transcription units¹⁰. Such repetitive sequences are also — though not invariably — found around, and sometimes even within, structural genes¹¹⁻²⁰. Much interest has been focused on the possibility that the interspersed repetitive sequences detectable by standard renaturation methods might be regulatory elements. Some of these repeated elements may indeed function as such. The examples shown in the table suggest, however, that while there appear to be genomic repeats that perform exactly the proposed regulatory roles, they are of a different class, invisible to the classical methods of repetitive sequence analysis because of their short length and relatively high degree of mismatch.

Recent research implies that the repeated

Table 1 Coordinately regulated gene sets that share 5' repetitive sequence elements

Organism	Gene	Regulation	Consensus sequence	% Homology with consensus sequence	Position	Functional assay	Refs
Yeast	<i>his1</i>	Induced by amino acid starvation	A [†] GTGACTC*	89	-334, -319	Yes (<i>his4</i> , ref. 24; <i>his3</i> , ref. 2)	1, 2, 8, 24-26
	<i>his3</i>			78	-170, -73*		
	<i>his4</i>			89	-99, -33		
	<i>Trp5</i>			78	-195, -183, -139, -88		
					-190, -65		
<i>Drosophila</i>	<i>hsp70</i> (87A7)	Induced by heat shock	CTNGAATNTTCTAGA [†]	85 [‡]	-62	Yes (<i>hsp70</i> , ref. 3)	3, 27-29
	<i>hsp70</i> (87C1)						
	<i>hsp83</i>			92	-74		
	<i>hsp26</i>			85	-62		
	<i>hsp27</i>			54	-68		
	<i>hsp68</i>			69	-61		
	<i>hsp23</i>			62	-75		
	<i>hsp22</i>			85	-147		
Silkmoth (<i>A. polyphemus</i>)	Late chorion genes	Expressed during choriogenesis	T [†] ACGTGAA [‡] CTT [†] CTAT	80 [§]	-87	Not tested	30
	401						
	18						
	Middle chorion genes		TG [†] TANNNAA [†] T	58 [§]	-87		
	292						
	10						
Human	Proopiomelanocortin	Glucocorticoid-repressed	TNAAGAGGAANAAGACGACATGA [¶] CA - - - T - - - C - GAGA - - AT - - -	67	-486	Not tested for POMC or MMTV	4, 31, 32
Mouse	MMTV [#]	Glucocorticoid-induced	- C - - - A - - - A - - - - - - - - -	95	-392	Negative evidence for RGH ⁴	
Rat	Growth hormone	Glucocorticoid-induced	- T - - - - - T - - - - A - T - - - G	95	-392	($P < 7 \times 10^{-9}$)**	
Chicken	Conalbumin	Steroid-induced egg-white proteins	AAAAATGGGC [¶]	100	-140	Not tested	33
	Lysozyme			78	-142		
	Ovalbumin			89	-140		

Except for the silkmoth chorions and four of the *Drosophila* heat-shock genes (*hsp22*, 26, 27 and 23), the genes of each set considered are unlinked (this is of course not relevant to the three mammalian genes). The 5'-flanking and coding sequences are otherwise non-homologous, except for the common features of the eukaryotic promoter (TATAA and CAAT box sequences). Consensus sequences are derived from best alignments, with residues denoted N where less than 50% of examples display a predominant nucleotide at the given position. Consensus sequences are the same as those proposed by original authors, except where stated. The % homology of each example with the consensus sequence is computed, ignoring those residues denoted N. In all cases, even where the consensus sequence is reiterated, only the best match with the consensus sequence has been used for the calculation. Note that in several cases other than yeast (including the *hsp70* and *hsp68* genes of *Drosophila* and the lysozyme gene of the chicken), other sequences with significant homology to the consensus sequence occur in the 5'-flanking DNA. Position is with respect to the mRNA cap site (if known) or major 5' end deduced by S₁ nuclease mapping or primer extension. More than one entry indicates multiple occurrences of the sequence in the vicinity of the same gene. 'Functional assay' means DNA transformation assays showing that the consensus sequence is necessary for physiological induction.

* The consensus sequence derived by Hinnebusch and Fink⁸, from comparing all four genes listed here, differs slightly from that inferred by Struhl², comparing only *his3* and *his4* genes, and *his3* regulatory mutants. The consensus sequence and positions quoted here are those deduced by Hinnebusch and Fink.

† The third nucleotide of the consensus sequence, denoted as G by Pelham³, varies in 4/7 examples (when the best alignment is used for *hsp68*). It is therefore denoted here as N.

‡ The *hsp70* genes at 87C1 and 87A7 are apparently the product of a recent duplication (or correction) and for this analysis are treated as a single sequence. The 150 nucleotides to the 5' side of the cap site in these two genes show only 12 substitutions, with no gaps (see ref. 29).

§ Since only two chorion genes are compared in each case, the % homology is calculated as the proportion of invariant nucleotides between the consensus regions of the two genes. By contrast, the overall % homology over the 5'-flanking DNA, using the alignment proposed by the original authors³⁰, is 45% for the pair of late chorion genes and 39% for the pair of middle chorion genes.

¶ The sequences are from different organisms, so they are quoted in full. Nucleotides corresponding with the consensus sequence are denoted by a dash. The residue marked with a dot in the consensus sequence is absent from one of the sequences.

¶ A specific consensus sequence was not stated by original authors.

Mouse mammary tumour virus. The sequence indicated lies upstream of the viral RNA start site in the viral long terminal repeat.

** Approximate probability of chance occurrence of these sequences was calculated as follows (see ref. 34). N is the number of possible occurrences of a flanking sequence of length n found in a given gene, in a region L nucleotides long: $N = L - n$. P is the probability of random occurrence of a sequence matched at m/n sites, where each nucleotide has an equal 1/4 chance of occurrence: $P = [n!/(n-m)!m!](1/4)^m(1-1/4)^{n-m}$. C is the probability of finding the desired mismatched sequence in the specified region of a second gene: $C = 1 - e^{-PN}$. If the sequence occurs another time in the same region, that is, of a third gene, the desired probability is C^2 . For the genes responding to glucocorticoid, since the positions of the consensus sequence vary from -392 to -486, the calculation was carried out with $L = 100$, and $m = 16$, $n = 21$, the average degree of mismatch observed⁴. Various corrections might be applied to the estimate, for example, for the effect of the 1-nucleotide deletion in the MMTV sequence, or for the fact that in the first comparison a homology occurring anywhere in the 5' region would have been scored. Nonetheless the occurrence of this sequence by chance in this region of the 5'-flanking DNA of the three genes is very improbable. For the egg-white protein genes all the homologous sequences occur within the same 12-nucleotide region. For this calculation, $L = 12$, $m = 7$ and $n = 9$.

sequences revealed by renaturation methods are scattered throughout the genome because many or all of them have (or had) the capacity to transpose during evolution. This is an extremely interesting observation in considering the problem of explaining the evolutionary origin of new biological forms. A dominant process in evolution is likely to have been the creation of novel coordinately induced structural gene networks²¹⁻²³. One hypothetical mechanism for this process suggested

earlier²¹ is the 'diffusion' around the genome of transposable *cis*-regulatory sequences, and their occasional insertion at productive locations in the vicinity of structural genes. The demonstrated and the putative *cis*-regulatory sequences considered in this article affect these ideas directly. It is thus easy to imagine that transposable sequence elements could occasionally carry with them and deposit very short repeat sequences that in the right genomic environment might display

receptor functions. Or, as in the case of the mutant *his4* yeast revertants, such potentially active regulatory sequences must occasionally arise *de novo* within transposable repeat elements, simply as a consequence of a small number of fortuitous single-base pair mutations. □

The authors are at the California Institute of Technology, Pasadena, California 91125. Research from this laboratory was supported by NIH grant GM-20927. H. T. J. is supported by a NATO-SRC fellowship.

Conversion of RNA to DNA in mammals: *Alu*-like elements and pseudogenes

Phillip A. Sharp

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

*Striking similarities between a subclass of mammalian pseudogenes and the *Alu* family of short repetitive DNA sequences which are dispersed throughout the mammalian genome, suggest that both are created by integration into the genome of DNA copies of RNA transcripts.*

DISPERSED throughout the mammalian genome are 100–500,000 short repetitive DNA sequences, distinguished from other repetitive sequences in that they are substrates for the *Alu* restriction enzyme and so commonly referred to as *Alu*-like elements (see ref. 1 for review). These elements range in length from 135 base pairs (bp) in the mouse to 300 bp in man and have long consecutive runs of deoxyadenosine [poly(dA)] at their 3' ends. Sequences in the 5' portion of a group of elements are about 80% homologous within a species and less homologous to elements in other species. Interestingly, *Alu*-like elements contain significant sequence homology to the small cytoplasmic 7S and 4.5S RNAs (see below), but it is two other features of *Alu*-like elements that have suggested that they have dispersed by replication through an RNA intermediate². The most suggestive feature is that most elements are flanked by direct repeats of sequences 7–20 nucleotides long. Flanking direct repeats are hallmarks of sequences that have been integrated in the genome, the repeats being formed by replication from staggered nicks at the site of insertion³. The origin of the integrated *Alu*-like elements is suggested by the fact that many are transcribed by RNA polymerase III, which initiates transcription at a site immediately downstream from the direct repeat and reads through the second direct repeat, yielding a product co-linear with the integrated element⁴.

It should be noted that, as a consequence of RNA polymerase III promoter sequences being contained within the transcribed region⁵, the transcripts of *Alu*-like elements will retain promoter sequences and DNAs derived from these transcripts should themselves be templates for RNA polymerase III. The genetic mobility thereby afforded genes transcribed by RNA polymerase III is not shared by those which are transcribed by RNA polymerases I and II, both of which have promoter sequences which are not themselves transcribed.

Although many *Alu*-like elements are good templates for *in vitro* transcription by RNA polymerase III, evidence suggests that most of the 500,000 human cellular sequences are not transcribed *in vivo*. Presumably this is because either particular flanking sequences are essential for their active transcription *in vivo* or because a factor solubilized in the *in vitro* extract is not available *in vivo*, or a component suppresses their transcription *in vivo*. It is difficult to reconcile the last possibility with the early findings that sequences of *Alu*-like elements are transcribed as part of heterogeneous nuclear RNA⁶. Recently, Young *et al.*⁷ have shown that an *Alu*-like element, when amplified on a simian virus 40 (SV40) vector in monkey cells, is transcribed by RNA polymerase III. Thus, these elements can be transcribed *in vivo* as well as *in vitro*. (Note that, as *Alu*-like elements typically lack termination sites, their transcripts should extend into flanking downstream sequences. How

such transcripts would be processed or degraded has not been studied.)

The recent discovery that the small cytoplasmic 7S RNA is part of a ribonucleoprotein (RNP) particle containing at least six different proteins suggests another possible function for *Alu* sequences⁸. This ribonucleoprotein functions in protein translocation across and protein integration into the endoplasmic reticulum membrane and has been named the signal recognition particle. About 100 nucleotides at the 5' end and 50 nucleotides at the 3' end of the 300-nucleotide 7S RNA are ~80% homologous to the consensus *Alu* sequence⁹ and perhaps it is through its *Alu*-like sequences that 7S RNA is bound to one or more of the six RNP proteins. If so, it is also possible that similar complexes are formed in the nucleus between proteins and the 1–5% of heterogeneous nuclear RNA sequences that contain *Alu* elements derived from introns and untranslated regions⁶. Perhaps removal of these protein-bound sequences by RNA splicing is necessary before mRNA is released to the cytoplasm. In fact, these protein–RNA interactions might be crucial for processing and transport of RNA. Thus duplication of *Alu* elements by insertion could provide a means of restricting RNA sequences to the nucleus.

Pseudogenes

Pseudogenes are partial duplicates of structural genes, incorporating sufficient changes that they are not biologically active and, in most cases, are not transcribed into RNA. For one subclass of pseudogenes, as for *Alu*-like elements, there is evidence to suggest that RNA intermediates were used as templates in their creation. In particular, (1) in the pseudogene the intervening sequences of the expressed gene have been precisely removed as when messenger RNA is produced by splicing; (2) a run of deoxyadenosine residues is found at the 3' end of the pseudogene in a position equivalent to the poly (A) on mRNA; and (3) in some cases sequence homology between the expressed gene and the pseudogene ends at the termini of the mRNA. Pseudogenes having some or all of these characteristics have been referred to as processed genes, recognizing that the information for constructing them was derived from processed RNA transcripts and not directly from existing chromosomal DNA segments¹⁰. Processed genes have been described for mouse α -globin¹¹, human κ immunoglobulin¹⁰, human β -tubulin¹², human metallothionein¹³ and rat α -tubulin¹⁴ but only in the last two examples do all three characteristics coexist.

These processed genes have another feature that relates to *Alu*-like elements: each is flanked by a direct repeat that varies in length from 10 to 20 bp. The best examples are the human metallothionein and α -tubulin pseudogenes, where one direct

repeat is positioned immediately upstream from the sequence corresponding to the 5' end of the mRNA and the second is positioned immediately downstream of deoxyadenosine sequences corresponding to the 3' end of the mRNA.

Pseudogene sequences have previously been characterized for genes encoding non-messenger RNA such as the small nuclear RNAs U1, U2 and U3. These pseudogenes are also commonly flanked by direct repeats, one member of which is precisely positioned adjacent to sequences complementary to the 5' terminus of the RNA. Van Arsdel *et al.*¹⁵ have proposed that these were also produced through a RNA intermediate.

The difficult question is, why does this process exist? Is it simply to reproduce *Alu*-type elements and, occasionally, to incorporate a mRNA in the process? Alternatively, the process might provide a pathway for converting information available as RNA sequences into potential genetic information. Duplication of genes has long been viewed as an important step in generating genetic information for novel functions. However, in the case of a pseudogene, the duplicated genetic material would be lacking in signals necessary for expression and could only be used for the development of novel genes by recombination with an active locus. There are some obvious advantages of restricting the duplication to genetic material to sequences found in RNA. First, the sequence would have a high probability of possessing useful information as its origin would be messenger RNA or structural RNA. Second, the inserted sequence would have no signals that might interfere with the expression or regulation of other genes. For example, the sequences should contain neither splicing signals nor sequences that regulated transcription by RNA polymerase I or II.

It has been demonstrated that retroviruses containing oncogenes have recombined with cellular sequences and replicated cellular RNA sequences during passage. Intervening sequences are frequently spliced from the acquired cellular sequences¹⁶ and it has been suggested that this provides a paradigm for the formation of processed pseudogenes. It is possible that examples of pseudogenes without introns will be discovered that were duplicated by retroviruses; these should be flanked on one or both sides by retroviral sequences. However, the recently characterized processed pseudogenes do not seem to be embedded in retroviral sequences, arguing against recombination with retrovirus as a step in their creation.

Mechanisms

If *Alu* dispersed elements and processed pseudogenes are generated from an RNA intermediate, what enzymes account for the process? The pathway probably involves two distinct steps: conversion of RNA sequences to DNA sequences and integration of these sequences by a transposase-type mechanism that produces varying lengths of direct repeats. The only characterized biochemical process for copying DNA from RNA is catalysed by viral reverse transcriptase. It is reasonable to suggest that a similar enzyme is responsible for the pathway discussed here. Indeed, given the abundance of *Alu* dispersed elements in mammalian cells, it is possible that the original function of

reverse transcriptase was duplication of these elements and that retroviruses have used the pathway and the enzyme for their own means. The mechanism of replication of retroviruses is well established and involves a tRNA primer, the jumping of a short primed oligonucleotide from one end of the RNA genome to the other and subsequent initiation of the second strand at a particular site (see ref. 17 for review). The DNA product is probably a linear duplex that integrates with duplication of 4–6 nucleotides at a chromosomal site (each virus duplicates segments of unique length). It is not obvious how a similar process could exactly copy a RNA for subsequent colinear insertion.

The RNA substrate, a RNA polymerase III product, which is reverse transcribed in the creation of *Alu* dispersed elements has not been identified. It is probably not the abundant 4.5S or 7S cytoplasmic RNA species. Although related in sequence⁹, no *Alu* dispersed element has been characterized that is identical to these RNAs. No other characterized small abundant cellular RNA seems to be a reasonable candidate. Thus, the template RNA is either heterogeneous in sequence and length, present at low levels, or synthesized only in a particular tissue.

Processed genes and *Alu*-like elements are heritable; thus these segments are inserted into DNA in cells of preimplantation embryos or in cells of germ tissue. Interestingly, evidence for induction of expression of endogenous retroviral information is well known for the 2-, 4-, and 8-cell stages of the preimplantation embryos. Electronmicroscopy of sections of these cells shows large numbers of intracisternal A particles. These particles are stained by antibodies to purified A-type particles from plasmacytoma cells¹⁸. Whether reverse transcriptase and an integration function are synthesized in the cells as a result of induction of retroviral functions has not been established.

It is attractive to propose that the human *Alu*-like elements have been continuously created from a small number of founder segments since man and mouse diverged. This would provide an explanation for the species-specific homology of *Alu* dispersed segments. (Gene conversion could also have contributed to maintenance of their relatedness but, as yet, there is no clear evidence of the frequency of this process in mammals.) If all 500,000 human *Alu*-like elements were generated since man and mouse diverged some 65 Myr ago and if they were selectively neutral, then a minimum estimate of their insertion frequency is $\sim 0.01 \text{ yr}^{-1}$, 1/1,000 the frequency of neutral or silent nucleotide changes¹⁹. Thus duplication and insertion of these segments may not constitute a significant mutation liability. Continuous insertion of new *Alu* dispersed elements might promote deletion of cellular sequences by homologous recombination. Instability of DNA sequences flanked by repetitive elements has been described²⁰. As only 1% of mammalian DNA is thought to encode sequences expressed as protein, most of the remaining 99% may be functionally neutral for insertions and deletions, and, in fact, may be derived and maintained by a continuous flux of such events.

I thank my colleagues at MIT for many helpful comments.

- Schmid, C. W. & Jelinek, W. R. *Science* **216**, 1065–1070 (1982).
- Jagadeeswaran, P., Forget, B. G. & Weissman, S. M. *Cell* **26**, 141–142 (1981).
- Cold Spring Harb. Symp. quant. Biol. **45** (1981).
- Elder, J. T., Pan, J., Duncan, C. H. & Weissman, S. M. *Nucleic Acids Res.* **9**, 1171–1189 (1981).
- Sakonju, S., Bogenhagen, D. F. & Brown, D. D. *Cell* **19**, 13–25 (1980).
- Jelinek, W. R. *J. molec. Biol.* **115**, 591–604 (1977).
- Young, P. R., Scott, R. W., Hamer, D. H. & Tilghman, S. M. *Nucleic Acids Res.* **10**, 3099–3116 (1982).
- Walter, P. & Blöbel, G. *Nature* **299**, 691–698 (1982).
- Ullu, E., Murphy, S. & Melli, M. *Cell* **29**, 195–202 (1982).
- Hollis, G. F., Hieter, P. A., McBride, O. W., Swan, D. & Leder, P. *Nature* **296**, 321–325 (1982).
- Nishioka, Y., Leder, A. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2806–2809 (1980).
- Wilde, C. D., Crowther, C. E., Cripe, T. P., Lee, G.-S. & Cowan, N. J. *Nature* **297**, 83–84 (1982).
- Karin, M. & Richards, R. I. *Nature* **299**, 797–802 (1982).
- Lemischka, I. R. & Sharp, P. A. *Nature* **300**, 330–335 (1982).
- Van Arsdel, S. W. *et al. Cell* **26**, 11–17 (1981).
- Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. *Cell* **22**, 777–786 (1980).
- Varmus, H. E. *Science* **216**, 812–820 (1982).
- Kelly, F. & Condamine, H. *Biochim. biophys. Acta* **651**, 105–141 (1982).
- Perler, F. *et al. Cell* **20**, 555–566 (1980).
- Calabretta, B., Roberson, D. L., Barrera-Saldana, H. A., Lambrou, T. P. & Saunders, G. F. *Nature* **296**, 219–225 (1982).

Pyroxene whiskers and platelets in interplanetary dust: evidence of vapour phase growth

J. P. Bradley & D. E. Brownlee

University of Washington, Department of Astronomy, Seattle, Washington 98195, USA

D. R. Veblen

Department of Earth and Planetary Sciences, The Johns Hopkins University, Baltimore, Maryland 21218, USA

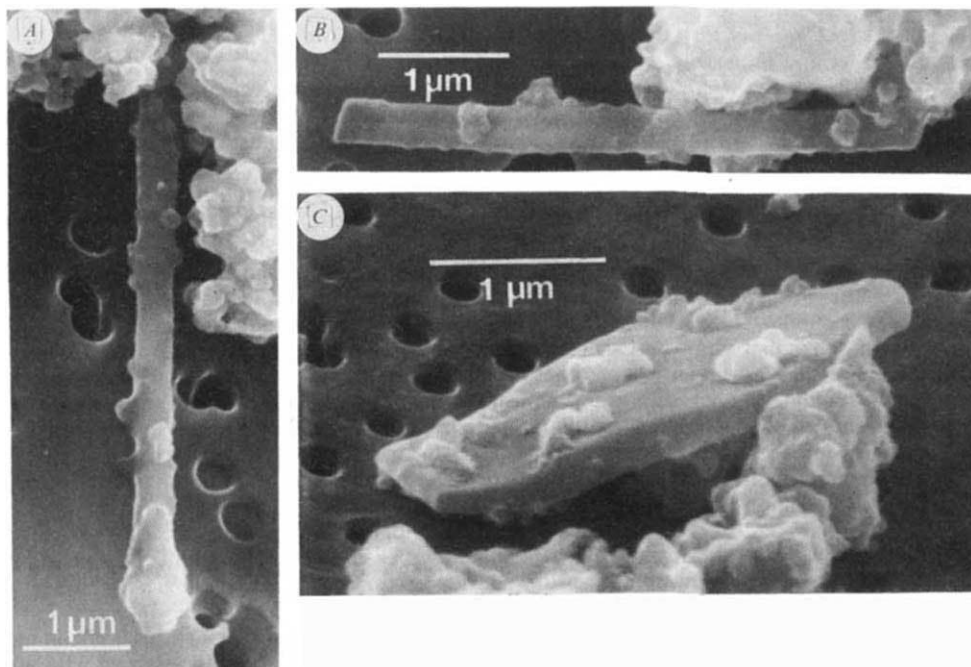
Enstatite (MgSiO_3) whiskers and platelets have been observed in interplanetary dust particles collected from the stratosphere. Their unique crystal morphologies and microstructures, such as axial screw dislocations, strongly suggest that they are primary vapour phase condensates which could have formed either in the solar nebula or in presolar environments.

GAS-TO-SOLID condensation is the fundamental mechanism that produces interstellar grains in the Galaxy and is a process which presumably had a role in the formation of planets from the solar nebula. Laboratory studies of preserved condensates could provide valuable insight into condensation processes and yet previous investigations of extraterrestrial materials have not yielded definitive identification of unaltered primitive condensates. Among other complications, the most primitive, carbon-rich meteorites show evidence of significant aqueous alteration and preservation of original condensates within these objects is in doubt^{1,2}. We report here strong evidence, from electron microscopic studies, for the discovery of unaltered primary condensates in interplanetary dust collected from the stratosphere. These micrometeorites (also known as Brownlee particles) are a type of extraterrestrial material that has only recently been available for laboratory investigations³. The grains in question are crystals of enstatite (MgSiO_3) that were found in a class of micrometeorite we call CP (chondritic porous). Among interplanetary dust types, CPs are the most conspicuously different from other extraterrestrial materials in

that they are fragile, highly porous aggregates of mostly sub-micrometre grains. Their extreme porosity suggests that they may be derived from contemporary comets. Comets are unique small bodies which formed at low temperatures and have been isolated at extreme distances from the Sun for most of their lifetimes. Of Solar System bodies, they are the most likely to contain well preserved samples of the primary particulate matter that formed the outer planets. These particles could include condensates from the solar nebula and pre-solar interstellar grains.

The samples were collected from the stratosphere at an altitude of 20 km by impaction onto silicone oil-coated plates mounted on U-2 aircraft, operated by the Airborne Science Division of the NASA Ames Research Center⁴. Assuming that the analysed particles had histories typical of the 10^4 tons of interplanetary dust that annually enter the atmosphere, then they would have experienced the following: (1) long-term storage inside a cometary or asteroidal parent body; (2) liberation from the parent body and a brief ($<10^5$ yr) existence as small interplanetary particles; (3) brief aerodynamic heating

Fig. 1 Scanning electron micrographs of the three pyroxene morphologies. *A*, an enstatite rod; *B*, an enstatite ribbon; *C*, an unusually large and thick enstatite platelet.



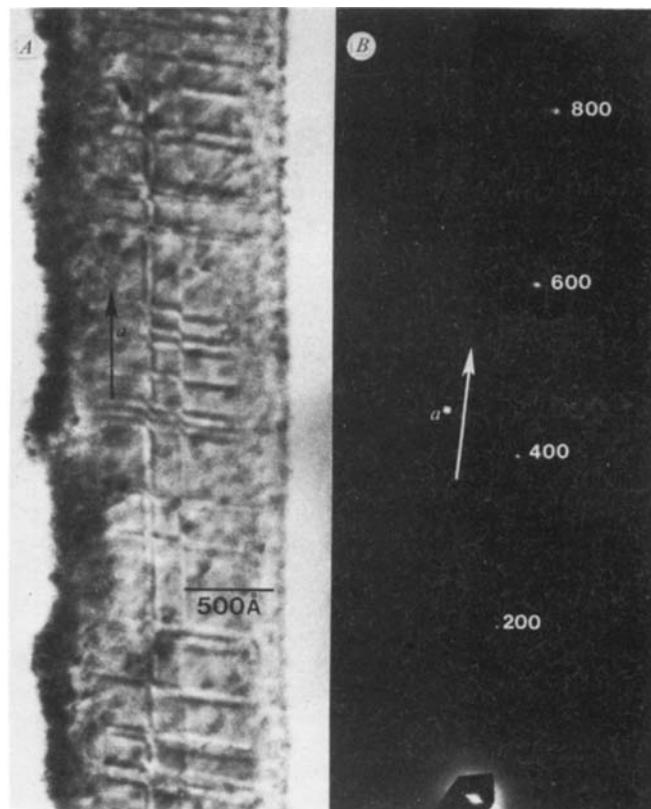


Fig. 2 A, bright-field transmission electron micrograph of a segment from a clinoenstatite rod viewed down the b crystallographic axis. The two features parallel to the axis of the rod are screw dislocations, and the features cutting across the rod are (100) stacking faults. B, a selected area electron diffraction pattern showing the ($h00$) reciprocal lattice row from this rod. The side bands (splitting) of the diffractions (especially visible for the 600 and 800) are probably related to the helical lattice distortions arising from the dislocations.

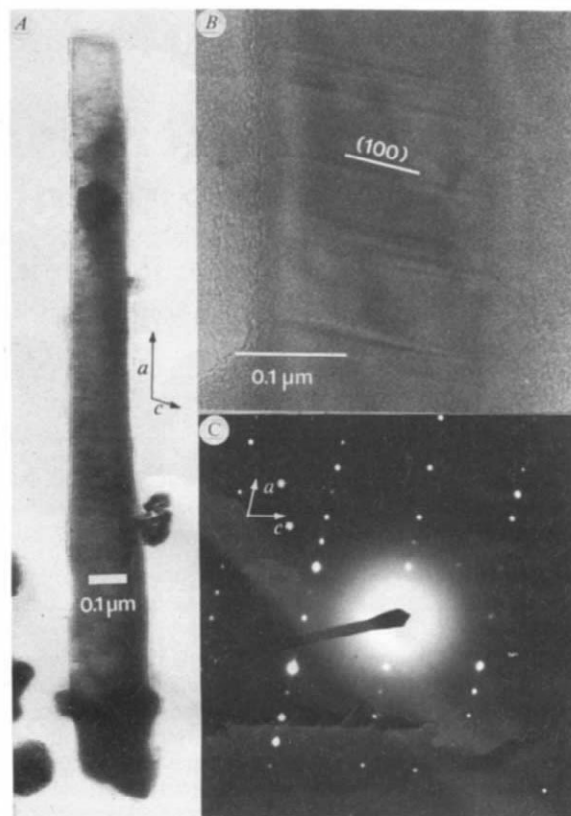


Fig. 3 A clinoenstatite ribbon viewed down the b crystallographic axis. A, an overview in bright-field; B, higher magnification image showing contrast arising from (100) stacking faults (contrast on either side of the ribbon is from the specimen support film); C, selected area electron diffraction pattern characteristic of untwinned clinoenstatite. Minor streaking parallel to a^* is due to the (100) stacking faults.

during entry into the atmosphere at 100 km altitude; (4) free fall in the atmosphere for a week or more before reaching the 20 km collection altitude.

All of the samples described here contained unmelted, angular FeS grains, indicating that the entry temperature pulse was $<1,000^\circ\text{C}$. Once the collection plates were returned to the laboratory, particles were located by conventional light microscopy as dark specks (typically $5\text{--}25\ \mu\text{m}$) embedded in silicone oil. Selected particles were removed from the oil, cleaned with solvents, and portions of them were mounted on appropriate substrates for chemical, morphological and structural analyses by scanning and transmission electron microscopy.

The most common micrometeorites collected in the stratosphere are fine-grained, carbon-rich materials with elemental compositions similar to CI chondrites, but $<20\%$ of these comprise the CP subgroup. The CPs often disintegrate during collection and handling, and are sometimes found on collection surfaces as regions of micrometre and sub-micrometre sized debris covering an impact area up to $300\ \mu\text{m}$ in diameter. Their estimated pre-impact densities range from ~ 1.5 to $<0.1\ \text{g cm}^{-3}$.

As CP micrometeorites are carbon-rich particles with chondritic elemental composition, they should be classified as a type of carbonaceous chondrite. However, CP material has not been found as a conventional sized meteorite, presumably because it is too fragile to survive atmospheric entry as large pieces. CP particles are distinct from conventional carbonaceous meteorites in porosity, morphology and mineralogy. For example, an important mineralogical difference relevant to this

study is the abundance of enstatite. Although enstatite is commonly observed in CP material, it is exceedingly rare in CI meteorites, the closest meteoritic analogue to CP particles.

Electron microscopic observations

Among the many mineral species that are observed within CP debris by analytical scanning electron microscopy (SEM) are enstatite crystals; this identification was confirmed by X-ray energy-dispersive analyses, electron diffraction and high-resolution lattice fringe imaging. These pyroxenes are ubiquitous, but volumetrically minor constituents of CP micrometeorites. Although we have observed many of these particles by SEM, difficulties in mounting them on suitable substrates has limited the total number of observations by transmission electron microscopy (TEM).

Instruments used for this study were, JEOL JSM-35C analytical scanning electron microscope equipped with a Tracor Northern TN-2000 X-ray energy-dispersive spectrometer; a JEOL 100CX transmission electron microscope, equipped with a Kevex X-ray energy-dispersive spectrometer and an electron energy-loss spectrometer; a JEOL 200CX high-resolution transmission electron microscope.

The enstatite particles are unlike the other micrometre-sized components in that some of them exhibit distinctive euhedral morphologies; we observed two whisker types (rods and ribbons) and platelets (Fig. 1). Rods have round or rectangular cross-sections and commonly have aspect ratios >20 . Ribbons are blade-shaped with aspect ratios of five or more; they have

previously been described by Fraundorf⁵. Platelets are flat, angular or disk-shaped crystals. Both the ribbons and platelets can occur as crystals thinner than 100 Å. These three enstatite morphologies are anomalous, as rock-forming terrestrial, lunar and meteoritic pyroxenes are generally not observed to possess such shapes.

Further departures from the properties of rock-forming pyroxenes were observed in the structural characteristics of the whiskers and platelets, which were investigated using a combination of electron diffraction and imaging, including lattice fringe imaging. Rods consist of essentially pure clinoenstatite and are grossly elongated in the crystallographic [100] direction (Fig. 2). (Rock-forming pyroxenes, when not equiaxial, tend to be elongated along [001].) Some of the rods also contain axial features that are consistent with screw dislocations. For example, in the enstatite whisker shown in Fig. 2, there are two dislocations running parallel to the whisker axis, and cutting across are parallel lines that result from (100) stacking faults (these have been identified by using lattice imaging techniques). The observed offset of the stacking faults as they cross the axial dislocations may be a result of both structural offset across the dislocations and electron optical effects arising from orientation and thickness variations in the whisker. Although the extremely small size of the rods made imaging experiments difficult, Burgers vectors determined by single-beam dark-field experiments⁶ on the rod of Fig. 2 demonstrated that the dislocations are pure screw in character.

Ribbons are blade-shaped whiskers that are also elongated along [100] (Figs 1B, 3). Their morphologies are superficially similar to those of many rock-forming pyroxene laths, except that rock-forming pyroxenes are never elongated along [100]. High-resolution lattice fringe images and electron diffraction data (including those of Fraundorf⁵) indicate that both the ribbons and rods consist exclusively of untwinned low clinoenstatite, together with isolated (100) stacking faults (Fig. 3). These observations are also anomalous; terrestrial clinoenstatite is almost invariably multiply twinned⁷, although one occurrence of an untwinned low-calcium clinopyroxene has been reported⁸. Likewise, meteoritic clinoenstatites that have been investigated are complexly twinned or consist of elaborate intergrowths of clino- and orthoenstatite^{9,10}.

Unlike the whiskers described above, platelets consist of flat, angular and disk-shaped crystals that are thin along [010] or [001] (Figs 1A, 4, 5). Although tabular mineral crystals are not uncommon, we know of no reported instances of pyroxene platelets exhibiting extreme flattening on either (010) or (001). The platelets also differ from rods and ribbons in that they consist of pervasively intergrown ortho- and clinoenstatite, together with material possessing extreme stacking disorder. Based on other occurrences of such intergrowths, it is likely that the platelets formed by inversion from protoenstatite during cooling⁹⁻¹¹.

Polymorphism and thermal conditions of growth

Ideally, the enstatite polymorph or polymorphs present in a crystal should clarify the thermal regime of growth. Atmospheric entry should not erase this information, because the thermal pulse is too brief to induce polymorphic changes in enstatite¹¹. Unfortunately, the phase relations of enstatite, although the subject of intensive study, remain poorly characterized¹¹⁻¹⁴. It is highly probable, however, based on the experiments of Smyth¹¹, Ojima¹⁴ and others that clinoenstatite is not a stable phase at any temperature in pressure conditions of 1 atmosphere or below and that protoenstatite and orthoenstatite are the stable high and low temperature forms, respectively. This view is taken in the following discussion.

The phase relations suggest that the clinoenstatite whiskers must have formed metastably either by inversion from protoenstatite or by metastable growth. The absence of multiple twinning in the clinoenstatite rods and ribbons would seem to rule

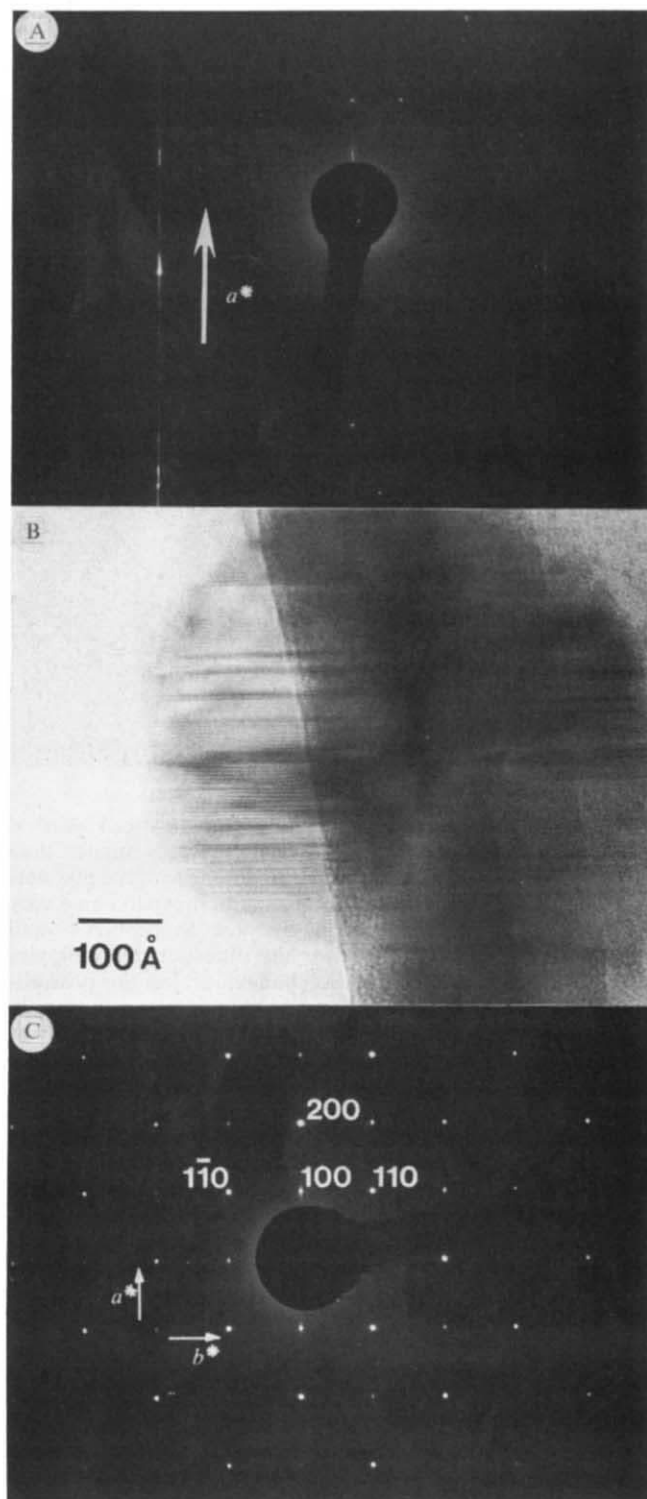


Fig. 4 A, a [010] selected area electron diffraction pattern of an enstatite platelet flattened on (010). Pronounced streaking parallel to a^* results from a mixture of enstatite polymorphs and extreme stacking disorder. B, bright-field electron micrograph of a disk-shaped enstatite platelet flattened on (010). This platelet lies on a thick carbon support with part of it extending over the edge. C, a [001] selected area electron diffraction pattern of an enstatite platelet flattened on (001). The $h+k$ = even diffractions are sharp, but the $h+k$ = odd diffractions are diffuse and streaked parallel to a^* as a result of stacking disorder.

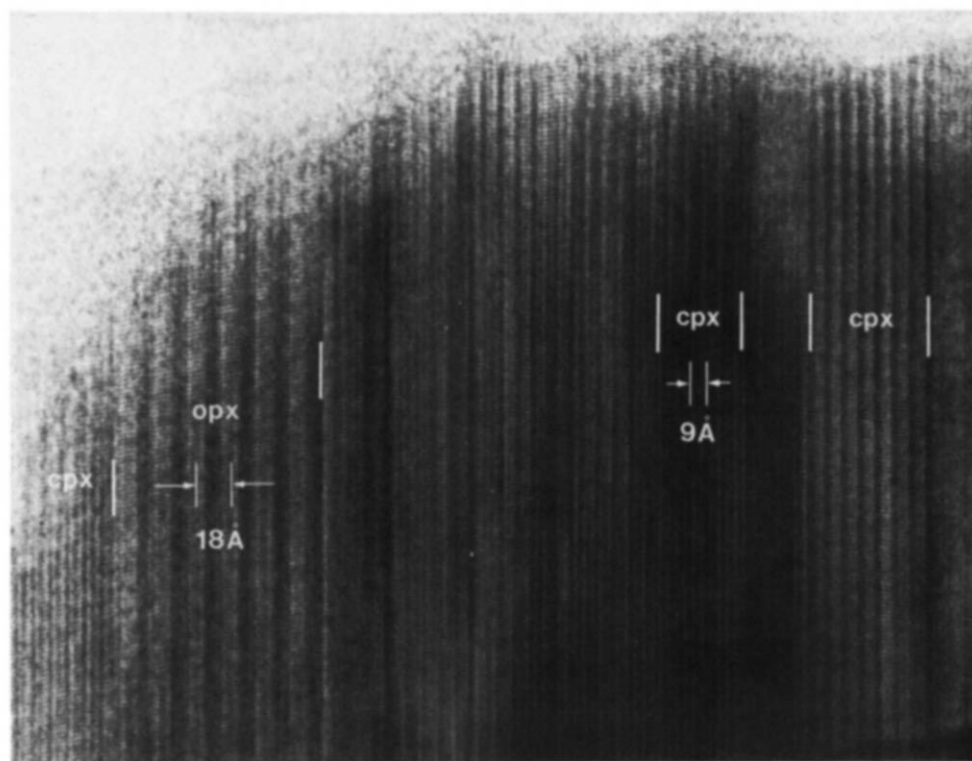


Fig. 5 High-resolution overview of the platelet shown in Fig. 4B, viewed parallel to [010] with the *c*-axis vertical. The platelet consists of fields of ordered clinoenstatite (cpx) and orthoenstatite (opx), as well as regions of pyroxene with stacking disorder (unlabelled). The 18 Å and 9 Å periodicities represent the (100) spacings for opx and cpx respectively.

out the first possibility, as such twinning is diagnostic of inverted protoenstatite. However, these crystals may be smaller than the expected twin domain size. On the other hand, the platelets do exhibit features typical of protoenstatite inversion on a very fine scale. It is conceivable that in these exceedingly small crystals, surface energy effects of the different morphologies could influence the transformation behaviour, but this possibility is not testable at present.

The possibility that the rods and ribbons grew metastably as clinoenstatite is, perhaps, more likely. Growth of metastable polymorphs is common, especially in cases of polytypism, as exhibited by enstatite. Spiral growth may have preferentially crystallized clinoenstatite whiskers in the protoenstatite stability field, while protoenstatite platelets formed by a different growth mechanism. We can place some constraints on this protoenstatite growth. Based on experiments of Smyth¹¹ and others, the low-temperature limit of protoenstatite stability is about 1,000 °C. The absence of orthoenstatite, other than as an inversion product of protoenstatite, strongly suggests that the thermal conditions of growth were above this temperature.

Kinetic aspects and mechanisms of whisker and platelet growth

Whiskers of at least 30 elements and many more compounds have been synthesized in the laboratory^{15,16}, and whiskers can also occur in nature¹⁷. A typical natural occurrence or synthesis experiment may produce a variety of crystal morphologies, including both whiskers (for example, rods and ribbons) and platelets¹⁷⁻¹⁹.

Crystal morphology can usually be explained in terms of crystal structure and symmetry^{20,21}. This is not the case with whiskers and platelets: to account for their shapes, it is necessary to consider other variables, such as symmetry of the growth medium and the presence of crystal defects^{17,22}. If any anisotropy that is not a part of the ideal bulk crystal structure (such as a screw dislocation) is introduced during crystal growth, gross morphological perturbations can occur, resulting in whiskers and platelets. Similarly, whisker growth by the vapour-liquid-solid (VLS) mechanism^{23,24} can be thought of as resulting from an asymmetrical growth environment.

Crystal defects can also dramatically affect growth kinetics. In some cases, the formation of new atomic layers as islands on a crystal face (two-dimensional nucleation) can control the growth rate²⁵. Below a critical level of supersaturation (of the order of 50% or greater²⁵), such nucleation does not occur at an appreciable rate. If a crystal contains a screw dislocation, however, it can grow at lower levels of supersaturation by a spiral mechanism, because such dislocations provide permanent, self-perpetuating growth ledges at the surface of a growing crystal (for example, see Fig. 1 of ref. 26). Because a crystal face containing an emergent screw dislocation can add new material much faster than other faces, extreme elongation into a whisker morphology can occur parallel to the dislocation, as first suggested by Sears²⁷.

The spiral growth mechanism for whiskers requires the presence of axial screw dislocations, while other mechanisms do not. Coexisting platelets and whiskers both with and without dislocations have been observed for other compositions, suggesting that multiple mechanisms can operate simultaneously to produce whiskers. Our observations on enstatite whiskers and platelets agree with those from other chemical systems. The rods that have been examined with TEM methods contain axial features consistent with spiral growth. On the other hand, the ribbons and platelets appear to be free of screw dislocations, suggesting that they formed by some other growth mechanism, presently unidentified.

Growth medium

Of critical importance for the interpretation of our results is identification of the likely growth medium for the enstatite whiskers and platelets. Did these crystals form by condensation from a gas phase, or is there an alternative explanation?

Although condensation from a vapour is the most common mode of growth, whiskers can form in other types of media, such as aqueous solutions²⁸. This is a most unlikely environment for CP micrometeorite formation, certainly not applicable to enstatite growth above 1,000 °C. Natural terrestrial whiskers commonly grow from supercritical hydrothermal fluids, again an unlikely environment for protoenstatite formation. Asbestos minerals can form hydrothermally or by solid-state reactions at high pressures in non-hydrostatic stress environments,

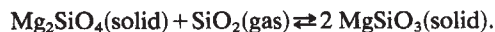
conditions that again are not applicable in the present case. Chain silicate asbestos also invariably is elongated parallel to [001], counter to the morphology of the enstatite whiskers.

Another possibility is that the whiskers and platelets formed by fragmentation of larger crystals. However, the cleavage and parting properties of pyroxenes would not produce the observed morphologies, such as whisker elongation parallel to [100]. Furthermore, terrestrial clinoenstatites and those from enstatite chondrules in meteorites are elongated parallel to [001]²⁹.

Growth from a relatively low-pressure vapour phase is the most likely remaining mode of formation for the enstatite whiskers and platelets. It is conceivable that they could have formed by a high-temperature gas-phase reaction with pre-existing olivine grains. However, such replacement reactions have not been demonstrated to form the observed range of morphologies, whereas primary growth from a vapour commonly does. In any case, the formation of the enstatite whiskers and platelets almost certainly involved crystallization in a hot gas. While it cannot be considered as rigorously proven, the hypothesis that is the most consistent with the structural and morphological observations is that the enstatite formed by primary condensation from a nebular gas cloud.

Donn and Sears³⁰ predicted that interplanetary dust derived from comets is likely to contain primordial whiskers and platelets formed by direct gas-to-particle condensation. We have identified enstatite crystals with these morphologies, strongly suggesting that condensation by whisker and platelet growth did have a role in forming primitive Solar System materials. Nuclei containing screw dislocations would have allowed equilibration of supersaturated vapour with a crystalline phase at supersaturations below those required for two-dimensional nucleation. Formation of whiskers and platelets by one-dimensional growth about screw dislocations would guarantee that supersaturation rapidly diminished after nucleation. Because equiaxial crystals might not be able to continue growing in the presence of whiskers and platelets, the condensed solids would consist predominantly of whiskers and platelets. If all the individual mineral grains in CP particles are pristine, first generation condensates, then it is significant that whisker morphologies are quite rare. Most of the mass of the CP particles is in the form of <0.1 µm polycrystalline grains, but it is possible that these smaller grains are simply aggregates of even smaller platelets.

One intriguing aspect of our whisker observations is that all of the grains with obvious whisker morphologies are almost pure enstatite. This may imply that of the abundant phases found in CP particles only enstatite could nucleate whiskers. Calculations of equilibrium condensation from a cooling gas with an initial solar composition indicate that enstatite, forsterite (Mg₂SiO₄ olivine), and FeNi alloy form over a narrow temperature interval³¹. The primary mechanism for formation of enstatite should be by reactions of solid forsterite with gas:



If equilibrium condensation of forsterite occurs and lowers the

Mg/Si ratio in the remaining gas, then only a small fraction of the enstatite could form by direct condensation. If forsterite formation does not change the gas composition then the two silicates condense essentially simultaneously³¹. With a non-solar Mg/Si gas ratio or with differential nucleation constraints, enstatite could form before forsterite. The observed whiskers and platelets, particularly those with screw dislocations, appear to have formed directly from vapour and not from a silicate precursor. Although olivine grains with variable iron content are common in all of the more than 300 chondritic composition micrometeorites that we have studied it is significant that not a single olivine whisker has been observed. In view of condensation theory it is also significant that metal occurs in only trace amounts within CP particles. The preservation of ultrathin enstatite platelets, with no evidence for low-temperature nebular equilibrium effects³¹, such as hydration or incorporation of substantial Fe²⁺, suggests that at least some fraction of original metal also should have survived.

Conclusions

Enstatite crystals with whisker and platelet morphologies have been observed within CP micrometeorites. They appear to be pristine condensates that have survived intact, perhaps from even before the earliest stages of Solar System formation. However, such crystals are apparently not abundant constituents of extraterrestrial materials. This may mean that very little material from the solar nebula or presolar environments formed by whisker growth mechanisms or that little of this material survived without later alteration or even complete recrystallization. Regardless of the specific origin of these enstatite whiskers and platelets within CP interplanetary dust, their presence and microstructures provide significant information about the role of crystal growth processes and particle formation. Further studies on these samples may provide direct data on those kinetic aspects of condensation which might influence the sequence of grain formation.

The observation of whiskers and platelets in particles of probable cometary origin, as predicted by Donn and Sears³⁰, confirms that such crystals may, indeed, have been important constituents of the early Solar System. Finally, we suggest that primary condensates in the form of whisker and platelet crystals of enstatite, as well as other minerals, may well be encountered in other primitive meteoritic materials. A search for such crystals, for example in the matrices of the less-altered chondrites of low metamorphic grade, might prove profitable.

This research was supported by NASA grant NSG-9052 and NSF grant EAR81-15790. High-resolution transmission electron microscopy was performed at the Facility for High Resolution Electron Microscopy, Arizona State University, established with support from the NSF Regional Instrumentation Facilities Program (Grant CHE79-16098). We thank L. Grossman and B. Donn for helpful discussions, also M. Wheelock, L. Fortson, D. Johnson, J. Wheatley and H. Kolar for assistance. Particle collections were made possible through the efforts of G. V. Ferry and the Airborne Science Division at NASA Ames Research Center.

Received 1 September; accepted 2 December 1982.

- DuFresne, E. R. & Anders, E. in *The Moon, Meteorites and Comets* (eds Middlehurst, B. M. & Kuiper, G. P.) 496-526 (University of Chicago Press, 1963).
- Bunch, T. E. & Chang, S. *Geochim. cosmochim. Acta* **44**, 1543-1578 (1980).
- Brownlee, D. E., Tomandl, D. A. & Olszewski, E. *Proc. 8th Lunar Sci. Conf.* 149-160 (1977).
- Brownlee, D. E. in *Cosmic Dust* (ed. McDonnell, J. A. M.) 295-336 (Wiley-Interscience, New York, 1978).
- Fraundorf, P. *Lunar planet. Sci.* **12**, 292-294 (1981).
- Hirsch, P. B., Howie, A., Nicholson, R. B., Pashley, D. W. & Whelan, M. J. *Electron Microscopy of Thin Crystals*, 222-246 (Butterworths, London, 1965).
- Deer, W. A., Howie, R. A. & Zussman, J. *Rock-Forming Minerals Ser. 2a*, 118 (Longman, London 1978).
- Trommsdorff, V. & Wenk, H. R. *Contr. Miner. Petrol.* **19**, 158-168 (1968).
- Iijima, S. & Buseck, P. R. *Am. Miner.* **60**, 758-770 (1975).
- Buseck, P. R. & Iijima, S. *Am. Miner.* **60**, 771-784 (1975).
- Smyth, J. R. *Am. Miner.* **59**, 345-352 (1974).
- Smith, J. V. *Miner. Soc. Am. Spec. Pap.* **2**, 3-29 (1969).
- Lindsley, D. H. *Miner. Soc. Am. Rev. Min.* **7**, 289-307 (1980).

- Ojima, M. J. *Japan Ass. Min. Petrol. Econ. Geol. Spec. Pap.* **3**, 97-103 (1982).
- Levitt, A. P. (ed.) *Whisker Technology* (Wiley-Interscience, New York, 1970).
- Nadgornyi, E. M. *Soviet Phys. Usp.* **5**, 462-477 (1962).
- Veblen, D. R. & Post, J. E. *Am. Miner.* (in the press).
- Drum, C. M. *J. appl. Phys.* **36**, 816-823 (1965).
- Drum, C. M. *J. appl. Phys.* **36**, 824-829 (1965).
- Donnay, J. D. H. & Harker, D. *Am. Miner.* **22**, 446-467 (1937).
- Hartman, P. & Perdok, W. G. *Am. Miner.* **41**, 449-459 (1956).
- Shafraunovskii, I. I. *Crystals of Minerals*, Vol. 1 (Leningrad University, 1957).
- Wagner, R. S. & Ellis, W. S. *Trans. metall. Soc. AIME* **233**, 1053-1064 (1965).
- Givargizov, E. I. *Curr. Topics Mater. Sci.* **1**, 79-145 (1978).
- Burton, W. K., Cabrera, N. & Frank, F. C. *Phil. Trans. R. Soc. A* **243**, 299-358 (1953).
- Frank, F. C. *Discuss. Faraday Soc.* **5**, 48-54 (1949).
- Sears, G. W. *Acta metall.* **3**, 361-366 (1955).
- Amelinckx, S. in *Growth and Perfection of Crystals* (eds Doremus, R. H., Roberts, B. W. & Turnbull, D.) 139-153 (Wiley, New York, 1958).
- Yasuda, M., Kitamura, M. & Morimoto, N. *Phys. Chem. Miner.* (in the press).
- Donn, B. & Sears, G. W. *Science* **140**, 1208-1211 (1963).
- Grossman, L. *Geochim. cosmochim. Acta* **36**, 597-619 (1972).

Light-chain movement and regulation in scallop myosin

Peter M. D. Hardwicke*, Theo Wallimann† & Andrew G. Szent-Györgyi*

* Department of Biology, Brandeis University, Waltham, Massachusetts 02254, USA

† ETH Eidgenössische Technische Hochschule, Institut für Zellbiologie, Honggerberg, CH-8093 Zürich, Switzerland

Photo-cross-linking techniques show that when scallop myosin or myofibrils are subjected to experimental conditions that cause relaxed muscles to go into rigor, the N-terminal portion of the regulatory light chain of myosin moves towards the essential light chain while the C-terminal portion stays in place. These changes occur on the myosin before combination with actin. Cross-linking of the N-terminal region to the essential light chain in rigor locks the myosin into a conformation such that calcium sensitivity of the actin-activated Mg-ATPase is lost.

CONTRACTION in molluscan muscles is regulated by calcium acting directly on myosin¹. The regulatory light chains (R-LCs) of myosin are essential for regulation. These myosin subunits (molecular weight (M_r) 17,000–20,000 (17–20K)) can be removed with EDTA, when the calcium dependence of the actin-activated Mg-ATPase activity of scallop myofibrils^{2–4} and of tension generation of skinned scallop fibre bundles⁵ is lost. Readdition of scallop R-LCs to desensitized myofibrils and fibre bundles restores calcium sensitivity. Desensitized myofibrils and fibre bundles combine readily with R-LCs from myosins of several different species and the hybrids are regulated if the source of the foreign R-LC is a regulated myosin, for example, *Mercenaria*, chicken gizzard^{6–8}. Hybrids formed with R-LCs of vertebrate striated myosin do not show calcium sensitivity^{6–8}. The R-LCs are elongated structures^{9–11} that occupy the distal half of the myosin head, extending towards the S2 region of the molecule^{12–14}. Loss of regulation produced by treatment with antibodies specific to essential light chains (SH-LC) of scallop myosin suggests that SH-LCs may also participate in regulation¹⁵. Protection of the thiol groups of the SH-LC by the R-LC indicates a close proximity of the two kinds of light chain¹⁶. Cross-linking studies in rigor solutions have shown that the SH-LCs and R-LCs overlap more than half of their length and are less than 9 Å apart¹⁷. We have now extended that approach so as to compare the relative position of the two light-chain types in media which cause skinned muscle fibres to relax, contract or go into rigor. The results indicate that the light chains move, that the movement is restricted for the most part to the N-terminal region of the R-LC, that it occurs on myosin before combination with actin, and that the light-chain movement is associated with the 'on' and 'off' states of myosin.

Experimental approach

Amino or thiol groups of R-LCs isolated from several different myosins were substituted in the dark with heterobifunctional photosensitive reagents. The modified R-LCs were then combined with scallop (*Aequipecten irradians*) myofibrils or myosin filaments whose own R-LCs had been previously removed with EDTA. The myofibrils were photolysed in relaxing (Mg-ATP, $<10^{-8}$ M Ca^{2+}) and rigor (no ATP, $\pm 10^{-4}$ M free Ca^{2+}) solutions. Myosin filaments were also photolysed in the presence of both Mg-ATP and calcium, a solution that activates fibre bundles. Photolysed and unilluminated control samples were electrophoresed on SDS-polyacrylamide gels, electrophoretically blotted onto nitrocellulose and subsequently analysed with the aid of specific anti-scallop R-LC and anti-scallop SH-LC IgGs¹⁸. Because only the R-LCs carry cross-linker, the presence of scallop SH-LCs in higher molecular weight complexes after photolysis demonstrates cross-linking between R-LCs and SH-LCs. The calcium sensitivity of the ATPase of the

hybrid myofibrils and myosins was also measured both before and after photolysis.

The position of cysteine residues in the primary sequence varies among the different R-LCs. *Mercenaria* R-LC is known to possess a single cysteine residue, ~50 residues from the N-terminus (out of a total of ~150)¹⁹. Chicken gizzard R-LC has a single cysteine at position 108 (out of a total of 171)²⁰, and rabbit R-LC two cysteines at residues 129 and 157 (out of a total of 169)²¹, towards the C-terminus. Use of these LCs modified with the thiol-directed photoactive agents *p*-azidophenacylbromide (*p*-APA) or benzophenone-4-maleimide (BPM) therefore results in well defined cross-linked products. The thiol groups are not essential for function³ and hybrids formed with the modified *Mercenaria* and gizzard R-LCs are regulated before photolysis. It is likely, therefore, that these light chains are positioned similarly to the native scallop R-LCs and can be used to follow the movement of distinct regions of the R-LCs. The Mg-ATPase^{6,7} and tension generation⁸ of hybrids formed with rabbit R-LCs are not calcium sensitive, so that these hybrids are not suitable for studying regulation, although they are useful in determining the extent of cross-linking of the C-terminal region in rigor and relaxing solutions.

Scallop R-LC contains no cysteine residues and was modified with *N*-hydroxysuccinimidyl-4-azidobenzoate (HSAB), which reacts with the ϵ -amino group of lysine. There are 15 lysine residues per mol scallop R-LC, and 4–7 of these were modified¹⁷. The positions of the substituted lysine residues are not known and different numbers and combinations of HSAB-labelled lysine residues are present in the R-LC population. Modified scallop R-LC was used only to study the effects of multiple cross-linking involving several portions of the R-LC.

Cross-linking and regulation in myofibrils

Cross-linking of R-LCs with SH-LCs and calcium regulation of the ATPase activity depended on the position of the cross-linker on the R-LC and on the conditions of photolysis. Modified *Mercenaria* R-LC cross-linked in rigor but not in relaxing solution (Fig. 1, Table 1), indicating that the region of the light chain about 50 residues away from the N-terminus changes its position relative to the SH-LC (Fig. 6). Most of the cross-linked SH-LC produced in rigor solution was found in a sharp band corresponding to a molecular weight of 35,000 consisting of one R-LC and one SH-LC (Fig. 1).

Modified gizzard R-LC and modified rabbit R-LC cross-linked to SH-LC both in rigor and in relaxing solution (Table 1, Figs 2, 3), although gizzard R-LC cross-linked more extensively in relaxing solution. The C-terminal part of the R-LC remains therefore close (<9 Å) to the SH-LC at rest and in rigor (Fig. 6).

Proximity of the different regions of the R-LC to the myosin heavy chains may be deduced from the formation of new bands

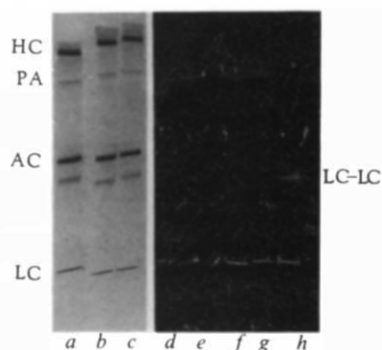


Fig. 1 Cross-linking of BPM-substituted *Mercenaria* R-LCs in scallop myofibrils. 10% SDS gels of: *a*, unilluminated samples; *b*, samples photolysed in relaxing solutions (40 mM NaCl, 2 mM MgATP, 1 mM EGTA, 1 mM MgCl₂, 5 mM phosphate, pH 7.0); *c*, samples photolysed in rigor solution (40 mM NaCl, 1 mM MgCl₂, 0.1 mM free Ca²⁺, 5 mM phosphate, pH 7.0). Blots of unilluminated samples: *d*, preimmune control IgG; *e*, scallop anti-R-LC IgG; *f*, scallop anti-SH-LC IgG. Blots of samples photolysed in relaxing solution (*g*) and rigor solution (*h*) treated with anti-scallop-SH-LC IgG.

Methods: Blots were developed with fluorescein isothiocyanate (FITC)-¹²⁵I-labelled goat anti-rabbit IgG. Sample loading was 50–60 µg per 5 cm. *Mercenaria* R-LC was prepared as described previously^{3,6}, and further purified on preparative urea gels. *Mercenaria* R-LC was reduced under N₂ in 0.1 M borate, pH 8.2, with a 30-fold molar ratio of dithiothreitol (DTT) to disulphide bonds and then passed through a 0.8×7 cm Biogel P-30 column in 0.2 mM DTT, 20 mM sodium acetate, pH 5.95. The reduced material was reacted for 1/2 h in 33% v/v dimethylformamide, 0.1 M HEPES, pH 7.5, with a 3-fold molar ratio of BPM (Molecular Probes Inc.) at room temperature under N₂ and the reaction then quenched by incubation for 1/2 h with a 10-fold molar ratio of DTT to BPM. Particulate material formed was removed by filtration through a 0.22-µm GS Millipore filter. After gel filtration on a 1.5×37 cm of G-25 (fine) Sephadex in 6 mM phosphate, pH 7.0, to remove reactants, the preparation was freeze-dried. Protein was determined by the method of Lowry *et al.*³¹, and the degree of labelling estimated to be 76% using $\epsilon_{280} = 16,450 \text{ M}^{-1} \text{ cm}^{-1}$ for the mercaptoethanol conjugate in 50 mM sodium phosphate, pH 7.0 (M. Lamkin and T. Tao, personal communication). Myofibrils were desensitized at 33 °C (ref. 4). Hybridization with the modified R-LC, photolysis, blotting of the proteins, staining with antibodies, quantitation of the cross-linking, ATPase measurements and gel electrophoresis were conducted as described previously¹⁷ with some modifications. To ensure a more complete transfer of the myosin heavy chains, 2% low melting point agarose containing 0.25 mg ml⁻¹ trypsin and 0.5 U ml⁻¹ activated papain was layered over the upper quarter of the slab gel a few minutes before blotting. The agarose faced away from the nitrocellulose sheets. As the isoelectric points of trypsin and papain are alkaline, the enzymes moved in the opposite direction to the muscle proteins and were not blotted. The limited proteolysis solubilized the myosin heavy chain such that only traces of the protein remained in the gel after blotting. HC, myosin heavy chain; PA, paramyosin; AC, actin; TM, tropomyosin; LC, light-chain.

in the 230,000-*M*, range consisting of cross-linked heavy chains and light chains. New high molecular weight bands were detected on Coomassie blue-stained gels following photolysis of myofibrils hybridized with rabbit R-LC¹⁷ and to a lesser extent in photolysed myofibrils hybridized with gizzard R-LC. The C-terminal region of the R-LC therefore lies close to the myosin heavy chains. Only very faint high molecular weight bands could be detected on the gels of photolysed myofibrils hybridized with modified *Mercenaria* R-LC (Fig. 1). The region of the light chain that is about 50 residues away from the N-terminus does not seem to be in direct contact with the heavy chain of myosin in rigor or at rest.

Calcium sensitivity of ATPase activity was reduced by cross-linking the N-terminal part of the R-LC to the SH-LC. Regulation of myofibrils containing modified *Mercenaria* R-LC was impaired by photolysis in rigor solution but remained unaffected

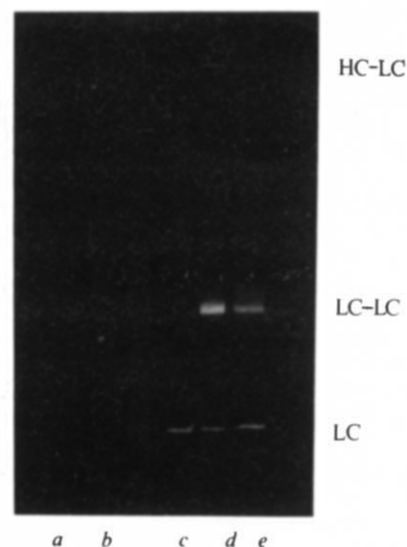


Fig. 2 Cross-linking of *p*-APA-substituted gizzard R-LCs in scallop myofibrils. Blots of unilluminated samples treated with: *a*, preimmune control IgG; *b*, scallop anti-R-LC IgG; *c*, scallop anti-SH-LC IgG. Blots of samples photolysed in relaxing (*d*) and in rigor (*e*) solutions treated with scallop anti-SH-LC IgG.

Methods: Blots were developed with FITC-¹²⁵I-labelled goat anti-rabbit IgG. Sample loading was 50 µg myofibrils per 5 cm. Chicken gizzard R-LC was prepared as described previously⁶ and then further purified by preparative gel electrophoresis. 10 mg of freeze-dried purified gizzard R-LC were dissolved in 1 ml 7 M GuHCl, 0.25 M HEPES, pH 7.5, and reduced with 5 mM DTT under N₂. MeOH was then added to 25% v/v (final) followed by *p*-APA (Pierce) in an equimolar ratio to total thiol and the reaction was allowed to proceed for 1/2 h at room temperature. After quenching with a 20-fold molar ratio of 2-mercaptoethanol to the *p*-APA, the preparation was dialysed against 5 mM sodium phosphate pH 7.0, then centrifuged at 1,000*g* and passed through a 0.22-µm GS Millipore filter to remove any precipitate before freeze-drying. The freeze-dried material was dissolved in 8 M urea, 10 mM phosphate, pH 7.0, and the preparation passed through a 1.2×16.7 cm Sephadex G-25 (fine) column in the same solvent. Following extensive dialysis against 5 mM phosphate, pH 7.0, the material was freeze-dried. After dissolving in water, the degree of labelling was determined to be 60% using $\epsilon_{300} = 2.01 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ in 2% v/v aqueous MeOH (ref. 32).

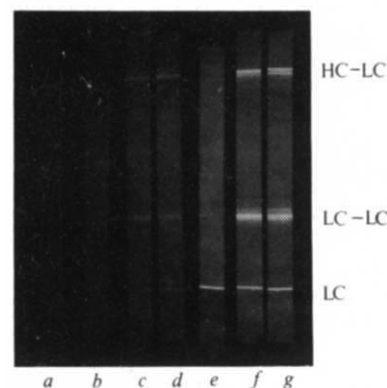


Fig. 3 Cross-linking with *p*-APA-substituted rabbit R-LC in scallop myofibrils. Blots of unilluminated samples treated with: *a*, preimmune control IgG; *b*, scallop anti-R-LC IgG. Blots of samples photolysed in relaxing (*c*) and in rigor (*d*) solutions and treated with rabbit anti-chicken R-LC IgG (gift of Dr Susan Lowey). Blots of unilluminated samples (*e*) and of samples photolysed in relaxing (*f*) and in rigor (*g*) solutions treated with scallop anti-SH-LC IgG. **Methods:** Blots were developed with FITC-¹²⁵I-labelled goat anti-rabbit IgG. Sample loading was 75–80 µg myofibrils per 5 cm. Rabbit R-LC was prepared according to standard procedures³. The purified R-LC was reacted with *p*-APA as described elsewhere¹⁷. Labelling was 59%.

by photolysis in relaxing medium (Table 1). The fall in calcium sensitivity was the result of an increase in ATPase activity in the absence of calcium, the ATPase activity in the presence of calcium being unchanged by cross-linking. The calcium sensitivity of myofibrils hybridized with modified gizzard R-LC remained unaffected by the cross-linking of R-LCs to SH-LCs or to heavy chains (Table 1).

The results with myofibrils containing modified scallop R-LCs (four to seven substitutions at unknown positions) could be readily explained by assuming the presence of cross-linkers both in the N-terminal and C-terminal regions. This modified R-LC cross-linked with SH-LC and myosin heavy-chain in rigor¹⁷ and to a lesser extent at rest. Regulation was impaired by photolysis in rigor solution (Table 1, Fig. 5). The presence or absence of calcium in the rigor solution during photolysis had no additional effects on cross-linking or on ATPase activity (Table 1). As the ATPase activity of scallop myofibrils hybridized with rabbit R-LCs showed no calcium sensitivity^{6,7}, regulation could not be studied with these hybrids, although the low ATPase activity⁷ was activated considerably by photolysis in both solutions (Table 1).

Light-chain movement in myosin filaments

Myosin filaments hybridized with modified *Mercenaria* R-LC were tested to see if light-chain movement required the presence of actin. Myosin filaments behaved similarly to myofibrils. Photolysis in the absence of ATP cross-linked R-LCs and SH-LCs extensively and impaired the calcium sensitivity of the actin-activated Mg-ATPase. Cross-links did not form and calcium sensitivity was not changed when myosin filaments were illuminated in a solution containing ATP but no calcium (Table

1, Fig. 4). The presence of actin is therefore not required for light-chain movement. This is consistent with absence of cross-linking between modified scallop or rabbit R-LCs and actin or tropomyosin in myofibrils either in relaxing or rigor conditions (Fig. 5). As myosin filaments in the absence of actin do not super-precipitate or contract, it is possible to explore the combined effects of ATP and calcium during photolysis. Photolysis in a solution containing ATP and calcium led to extensive cross-linking of R-LCs and SH-LCs accompanied by a significant loss of regulation (Table 1, Fig. 4). These results indicate that calcium in the presence of ATP causes the light chain to move.

General conclusions and implications

These studies establish that the N-terminal part of the R-LC moves relative to the SH-LC from a separation of less than 9 Å to one greater than this distance. The site of the conformational change, however, is unknown.

Hydrodynamic^{9,10} and X-ray scattering¹¹ studies indicate that the light chains are elongated structures, although the evidence that its conformation is retained when bound to the heavy chain is rather indirect^{15,19}. The fact that photosensitive reagents attached at different positions on the R-LC can cross-link with the SH-LC is consistent with such a picture and suggests specific interaction between these light chains in regulation. It is possible, however, that the light-chain rearrangements simply reflect the conformational changes of the heavy chain with which they are associated. The polarity of the R-LC defines whether the movable part is towards the S1-S2 hinge or entirely within the myosin head. The loss of an N-terminal peptide containing 10–20 residues from rabbit R-LC²², gizzard R-LC²³ or scallop

Table 1 Effects of photolysis on ATPase activity and cross-linking

	Unphotolysed control	Rest	Photolysed Rigor (Ca ²⁺)	Rigor (EGTA)
Myofibrils:				
HSAB-scallop R-LC (3)				
ATPase $\mu\text{mol min}^{-1} \text{mg}^{-1}$	0.29 $\pm$ 0.02	0.36 $\pm$ 0.11	0.35 $\pm$ 0.07	0.30 $\pm$ 0.07
ATPase ratio	0.05	0.05	0.45	0.43
% SH-LC cross-linked		46 $\pm$ 17	65 $\pm$ 7	63 $\pm$ 16
% R-LC cross-linked		85 $\pm$ 5	77 $\pm$ 12	74 $\pm$ 10
p-APA- and BPM- <i>Mercenaria</i> R-LC (3)				
ATPase $\mu\text{mol min}^{-1} \text{mg}^{-1}$	0.18 $\pm$ 0.04	0.20 $\pm$ 0.06	0.22 $\pm$ 0.09	
ATPase ratio (BPM-)	0.22	0.29	0.60	
% SH-LC cross-linked		<5	41 $\pm$ 4	34*
p-APA gizzard R-LC (5)				
ATPase $\mu\text{mol min}^{-1} \text{mg}^{-1}$	0.14 $\pm$ 0.01	0.17 $\pm$ 0.04	0.23 $\pm$ 0.03	
ATPase ratio	0.25	0.29	0.33	
% SH-LC cross-linked		64 $\pm$ 6	39 $\pm$ 10	
p-APA-rabbit R-LC (2)				
ATPase $\mu\text{mol min}^{-1} \text{mg}^{-1}$	0.04	0.13	0.23	
% SH-LC cross-linked		66	71	
% R-LC cross-linked		70	78	
Myosin:				
p-APA/BPM <i>Mercenaria</i> R-LC (3)				Activating
ATPase, $\mu\text{mol min}^{-1} \text{mg}^{-1}$	0.18 $\pm$ 0.06	0.20 $\pm$ 0.09	0.21 $\pm$ 0.10	0.24 $\pm$ 0.10
ATPase ratio (BPM-)	0.23	0.21	0.62	0.48
% SH-LC cross-linked		<10	39 $\pm$ 9	29 $\pm$ 4

ATPase activity in 20 mM NaCl, 1 mM MgCl₂, 2 mM MgATP, 0.2 mM CaCl₂, 0.1 mM EGTA and pure rabbit actin added in ratios of 0.5–1 w/w. Values for ATPase and % cross-linking are averaged (no. of different preparations shown in parentheses) as these do not depend greatly on the extent of R-LC uptake. ATPase ratios (ATPase in 0.1 mM EGTA/ATPase in 0.1 mM free Ca²⁺) are shown for a single preparation as the control values depend greatly on the uptake of R-LC. All preparations showed the same trend. In 11 *Aequipecten irradians* myofibril preparations ATPase activity averaged 0.37 $\pm$ 0.03 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ with ATPase ratios of 0.04 $\pm$ 0.03; following desensitization at 33 °C (ref. 4) the values were 0.19 $\pm$ 0.03 and 1.16 $\pm$ 0.09 $\mu\text{mol min}^{-1} \text{mg}^{-1}$. Three myosin preparations had ATPase activities of 0.38 $\pm$ 0.09 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ and ATPase ratios of 0.07 $\pm$ 0.05; after desensitization at 23 °C (ref. 15) the values were 0.14 $\pm$ 0.02 and 1.29 $\pm$ 0.22. Myofibrils and myosins were hybridized with modified R-LCs as described in the figure legends. Conditions of photolysis, measurements of ATPase activity and cross-linking were as described previously^{1,17}. In hybrids with modified gizzard and *Mercenaria* R-LCs the SH-LC cross-linked with myosin heavy chains in amounts <10% probably due to a lack of complete specificity of the cross-linking reagents. The amount of SH-LC-heavy-chain complex was the same when photolysed in 'relaxing' or 'rigor' solution and was not included in the values given in Table 1 for gizzard and *Mercenaria* R-LCs. Composition of relaxing (ATP without calcium), rigor (no ATP) and activating (ATP with calcium) solutions are as described in Figs 1 and 4.

* Single experiment.

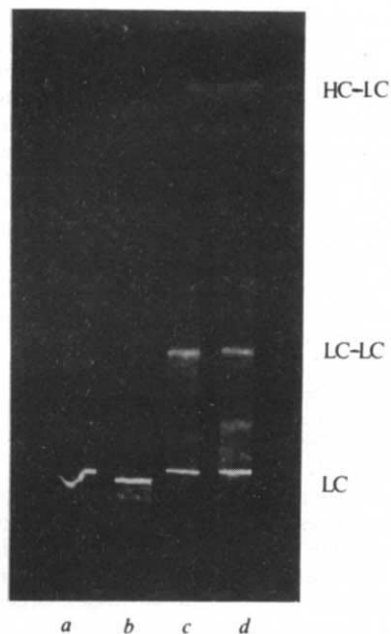


Fig. 4 Cross-linking with BPM-substituted *Mercenaria* R-LC in scallop myosin. Blots of unilluminated samples (a) and of samples photolysed in relaxing (b), rigor (c) and activating (d) (40 mM NaCl, 2 mM MgATP, 1 mM MgCl₂, 0.1 mM CaCl₂, 5 mM phosphate, pH 7.0) solutions. **Methods:** Blots were treated with scallop anti-SH-LC IgG and developed with FITC-¹²⁵I-labelled goat anti-rabbit IgG. Sample loading was 50–60 µg myofibrils per 5 cm. 76% BPM-labelled *Mercenaria* R-LC was used. Myosin was desensitized at 23 °C (ref. 15). Hybridization of myosin with *Mercenaria* R-LC and photolysis were conducted at low ionic strength in the same solutions as used with myofibrils.

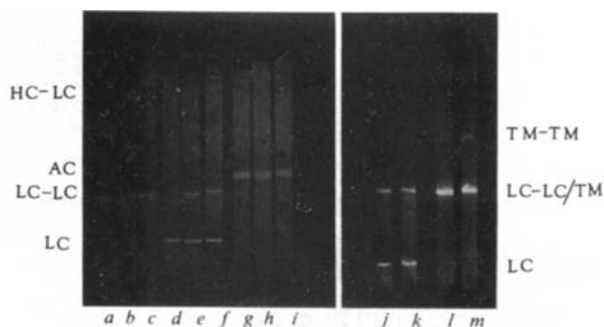


Fig. 5 Cross-linking of HSAB-substituted scallop R-LCs in scallop myofibrils. a–c, Blots treated with scallop anti-R-LC IgG; d–f, j, k, blots treated with scallop anti-SH-LC IgG; g–i, blots treated with rabbit anti-mouse actin IgG (gift of Dr Don Winkelmann); l, m, blots treated with rabbit anti-scallop tropomyosin IgG. a, d, g, Unilluminated samples; b, e, h, j, l, samples photolysed in relaxing solution; c, f, i, k, m, samples photolysed in rigor solution. **Methods:** Blots were treated with FITC-¹²⁵I-labelled goat anti-rabbit IgG with the exception of blots g–i which were treated with a mixture of 1,000× diluted FITC-labelled rabbit anti-mouse IgG (Miles) and 0.1 µCi ¹²⁵I-labelled rabbit anti-mouse IgG ml⁻¹ (NEN). Two different experiments with sample loading of 125 µg protein per 5 cm. HSAB-scallop R-LC was prepared as described elsewhere¹⁷. 4–6 mol of lysine were labelled per mol of protein. Note that anti-mouse actin-IgG is seen only in the position of the actin monomer, and anti-scallop tropomyosin IgG is detected only in the position corresponding to the monomeric tropomyosin chain, with only a very weak fluorescence at the tropomyosin dimer position that was also present in unilluminated samples. Fluorescent bands at positions corresponding to actin light chain or tropomyosin light chain complexes were not observed.

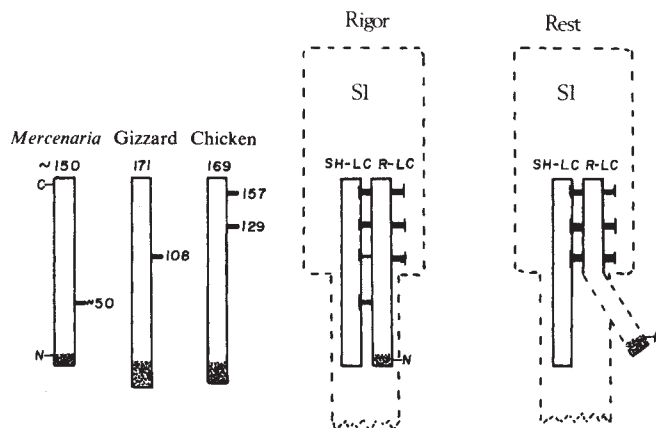


Fig. 6 A schematic diagram summarizing cross-linking results. a–c, Residues substituted by cross-linkers on *Mercenaria*, gizzard and rabbit R-LCs. Sequence homology indicates that the C-termini of the R-LCs are aligned^{20,23}. d, e, Cross-links at rest and in rigor between R-LCs and SH-LCs and between R-LCs and heavy chains. We assumed that the light chains are rod shaped, that the N-terminus of the R-LC lies towards S2, and that the site of the conformational change is on the R-LC. The position of the N-terminal part of the R-LC at rest is unknown. The shaded area on the R-LC represents the N-terminal peptide that is not required for function^{23,24}.

R-LC²⁴ during S1 preparation by proteolytic enzymes suggests that the N-terminus of the R-LC is oriented towards S2 and complements experiments on the important role of S2 in regulation²⁴; however, more direct evidence for the polarity is needed to choose between alternative models of light-chain function.

The interpretation of the findings in terms of light-chain movements depends on the assumption that most of the cross-links are formed between the SH-LC and R-LCs of the same myosin head. It is unlikely that there is significant inter-molecular cross-linkage of different myosin molecules, as substituted *Mercenaria* R-LCs do not cross-link in myofibrils or in myosin filaments in relaxing solution although in these conditions the mobility of myosin cross-bridges is greatly enhanced^{25,26}. We cannot at present exclude cross-linkage of R-LCs and SH-LCs derived from the two different heads of the same myosin molecule, but the effects of such interactions on regulation are likely to be of secondary importance because single-headed myosin is also regulated²⁴. The likelihood of this type of cross-linking is also reduced by the finding that in relaxing medium SH-LCs do not cross-link to modified *Mercenaria* R-LCs, although these are present on both heads.

The carboxyl end of the R-LCs seems to be of particular importance in the binding of light chains by heavy chains^{19,27}. Electron paramagnetic resonance studies of Bagshaw and Kendrick-Jones¹⁹ have also shown that on recombination of *Mercenaria* R-LCs with myosin heavy chains, a spin-label on the thiol group of the R-LC remained mobile, whereas those on rabbit R-LCs were extensively immobilized.

The findings fit the concept of protein dynamics of structural proteins, proposed by Caspar and Cohen²⁸, requiring the presence of both variable and conserved linkages. The conserved linkages could be situated in the region of carboxyl end of the R-LCs which seems to be permanently fixed. The position of the N-terminal portion of the R-LC depends on the physiological state and would be the site of the variable linkages. The lack of spectroscopic effect of Mg-ATP and calcium on regulated myosins suggests that conformational changes, if any, must be limited in scope and may involve restricted parts of the molecule²⁹. A hinge-like motion with the C-terminal part of the R-LC fixed and the N-terminal region of the R-LC movable would fit such observations, but the actual movement may be more complex. Clearly, a more detailed mapping of the R-LC movement with the use of additional foreign hybrids is necessary for a better description of how myosin subunits

reorganize in the different regulatory states of myosin. The lack of cross-linking of modified *Mercenaria* R-LC with SH-LC or the myosin heavy-chains in relaxing solution does not necessarily mean that the N-terminal end of the R-LC is completely free at rest. It is possible that a cross-linker closer to the N-terminus would facilitate the detection of interactions between regulatory light chains and heavy chains of myosin in relaxing solutions.

The movement of the light chains does not seem to require the presence of actin. The changes occurring on myosin therefore seem to determine its ability to interact with actin. Such a model of the 'on'-'off' switch fits the observation that myosin Mg-ATPase is activated severalfold by calcium even in the absence of actin³⁰. The fact that removal of calcium from rigor solutions has no effect on either cross-linking or regulation in myofibrils implies that calcium can regulate only the myosin-nucleotide complex.

Regulation of the actin-activated ATPase activity relates to cross-linking of R-LCs to SH-LCs in a rather simple manner. When the amino-terminal part of the R-LCs cross-links to the SH-LC, ATPase activity remains high even in the absence of calcium, that is, the regulatory switch is locked in the 'on' position. Cross-linking of the carboxyl-terminal part of the R-LC to SH-LC does not impair regulation. Evidently, some mobility of part of the amino-terminal region is required for a regulated myosin. One assumes that this mobility is not abolished when hybrids containing modified *Mercenaria* or modified scallop R-LCs are photolysed in relaxing solution. It is somewhat surprising that regulation is retained in myofibrils containing modified scallop R-LCs, as it is unlikely that the whole

N-terminal region of the R-LC remains freely mobile when interactions with actin are inhibited. However, it is not known where on the scallop-R-LC the cross-linkers are attached, and it is possible that the ϵ -amino groups nearer to, but not at the very N-terminal end of scallop R-LC are unreactive (clipped light chains lacking about 10 residues from the N-terminus are functional²⁴). One would predict that a cross-linker attached to a residue closer to the N-terminal end than the thiol of *Mercenaria* R-LC (somewhere between residues 10 and 50) might abolish regulation in two distinctly different ways: cross-linking in rigor to the SH-LC would fix the system in the 'on' state; in contrast, cross-linking to the heavy chain at rest would lock the system in the 'off' state.

At present the position of the R-LC at rest is unknown. There is no evidence that R-LCs are sufficiently close to actin or tropomyosin at rest or in rigor for cross-linking. R-LCs do not directly affect the ATPase site⁴ and there is no evidence that the R-LCs directly block actin binding sites. A steric hindrance model of regulation, however, is difficult to exclude until the position of the R-LC at rest is ascertained.

This research was supported by grants from PHS (AM 15963), the Muscular Dystrophy Association and the Swiss NSF (SNF 3.707-80 to Hans M. Eppenberger). We thank Dr Peter D. Chantler for discussions and for insisting that the cross-linking in relaxing solutions is a feasible experiment; Dr Eva M. Szentkiralyi and Dr Carolyn Cohen for discussions and for helping with the manuscript, Ms Izabela Plucinska for technical assistance, Dr Susan Lowey for the gift of the rabbit anti-chicken DTNB-LC IgG and Dr Don Winkelman for the gift of mouse anti-rabbit actin IgG.

Received 24 August; accepted 11 November 1982.

1. Kendrick-Jones, J., Lehman, W. & Szent-Györgyi, A. G. *J. molec. Biol.* **54**, 313-326 (1970).
2. Szent-Györgyi, A. G., Szentkiralyi, E. M. & Kendrick-Jones, J. *J. molec. Biol.* **74**, 179-203 (1973).
3. Kendrick-Jones, J., Szentkiralyi, E. M. & Szent-Györgyi, A. G. *J. molec. Biol.* **104**, 747-775 (1976).
4. Chantler, P. D. & Szent-Györgyi, A. G. *J. molec. Biol.* **138**, 473-492 (1980).
5. Simmons, R. M. & Szent-Györgyi, A. G. *Nature* **273**, 62-64 (1978).
6. Sellers, J. R., Chantler, P. D. & Szent-Györgyi, A. G. *J. molec. Biol.* **144**, 223-245 (1981).
7. Scholey, J. M., Taylor, K. A. & Kendrick-Jones, J. *Biochimie* **63**, 255-271 (1981).
8. Simmons, R. M. & Szent-Györgyi, A. G. *Nature* **286**, 626-628 (1980).
9. Stafford, W. F. & Szent-Györgyi, A. G. *Biochemistry* **17**, 607-614 (1978).
10. Alexis, M. N. & Gratzner, W. B. *Biochemistry* **17**, 2319-2325 (1978).
11. Hartt, J. E. & Mendelson, R. A. *Biophys. J.* **33**, 279a (1979).
12. Craig, R. *et al. J. molec. Biol.* **140**, 35-55 (1980).
13. Flicker, P., Wallimann, T. & Vibert, P. *Biophys. J.* **33**, 279a (1981).
14. Vibert, P. & Craig, R. *J. molec. Biol.* **157**, 299-319 (1982).
15. Wallimann, T. & Szent-Györgyi, A. G. *Biochemistry* **20**, 1188-1197 (1981).
16. Hardwicke, P. M. D., Wallimann, T. & Szent-Györgyi, A. G. *J. molec. Biol.* **156**, 141-152 (1982).

17. Wallimann, T., Hardwicke, P. M. D. & Szent-Györgyi, A. G. *J. molec. Biol.* **156**, 153-173 (1982).
18. Wallimann, T. & Szent-Györgyi, A. G. *Biochemistry* **20**, 1176-1187 (1981).
19. Bagshaw, C. R. & Kendrick-Jones, J. *J. molec. Biol.* **140**, 411-433 (1980).
20. Maita, T., Chen, J. I. & Matsuda, G. *Eur. J. Biochem.* **117**, 417-424 (1981).
21. Collins, J. H. *Nature* **259**, 699-700 (1976).
22. Bagshaw, C. R. *Biochemistry* **16**, 59-67 (1977).
23. Jakes, R., Northrop, F. & Kendrick-Jones, J. *FEBS Lett.* **70**, 229-234 (1976).
24. Stafford, W. F., Szentkiralyi, E. M. & Szent-Györgyi, A. G. *Biochemistry* **18**, 5273-5280 (1979).
25. Mendelson, R. A. & Cheung, H. *Science* **194**, 190-192 (1976).
26. Thomas, D. D., Ishiwata, S., Seidel, J. C. & Gergely, J. *Biophys. J.* **32**, 873-889 (1980).
27. Kendrick-Jones, J. & Jakes, R. in *Int. Symp. Myocardial Failure* (eds Ricker, G., Weber, A. & Goodwin, J.) 28-40 (Tagernsee, Munich, 1976).
28. Caspar, D. L. D. & Cohen, C. *Nobel Symposium Vol. 2* (eds Engstrom, A. & Strandberg, B.) 393-414 (1969).
29. Chantler, P. D. & Szent-Györgyi, A. G. *Biochemistry* **17**, 5540-5548 (1978).
30. Ashiba, G., Asada, T. & Watanabe, S. *J. biol. Chem., Tokyo* **88**, 837-846 (1980).
31. Lowry, D. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1956).
32. Hixson, S. H. & Hixson, S. H. *Biochemistry* **14**, 4251-4254 (1975).

Eukaryotic RNA polymerase II binds to nucleosome cores from transcribed genes

Bradford W. Baer & Daniela Rhodes

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Purified RNA polymerase II from calf thymus can bind to about 15% of the nucleosome core particles prepared from mouse myeloma cells, forming a discrete complex having a sedimentation coefficient of 18S. These bound nucleosome cores are heavily enriched in transcribed DNA sequences, are deficient in histones H2A and H2B, and undergo a reversible change in structure on RNA polymerase II binding.

RNA POLYMERASE II is responsible for the transcription of many genes coding for protein in the nucleus. The selective initiation, elongation and termination of transcription is to a large extent, of course, due to the specific nucleotide sequences of particular genes. But eukaryotic DNA occurs in the form of chromatin, complexed with histones and other chromosomal proteins in nucleosomes¹. This type of packaging can, in many

cases, include the portion of the genome containing transcribed genes^{2,3}. Therefore, RNA polymerase may encounter a chromatin template during transcription, raising additional possibilities for the mechanisms by which genes are selectively transcribed.

This notion has been approached experimentally in several ways. Chromatin has been subjected to various combinations

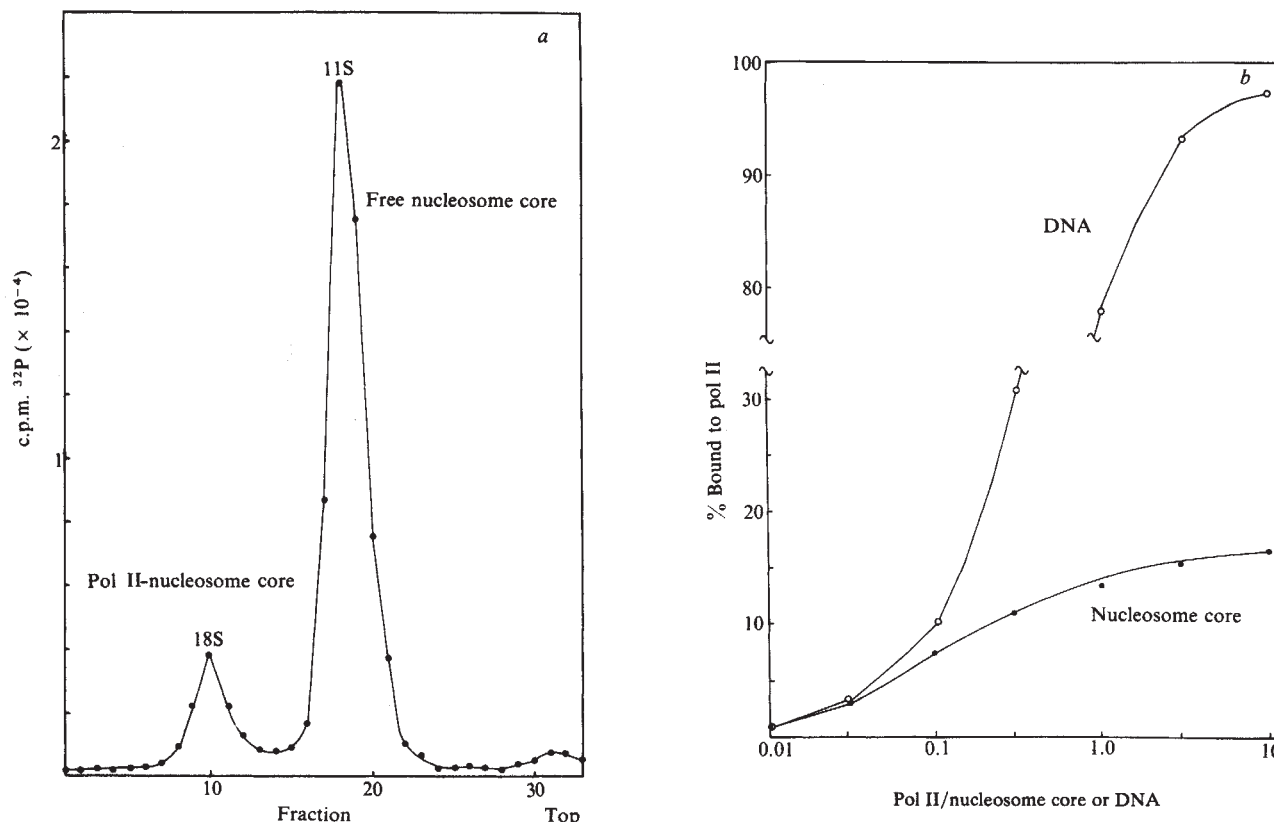


Fig. 1 Detection of an RNA polymerase II-nucleosome core complex by sedimentation. *a*, Sedimentation profile of a typical RNA polymerase II-nucleosome core binding mixture. *b*, Titration with nucleosome cores and 146-nucleotide pair DNA extracted from nucleosome cores. **Methods:** *a*, A 10-fold molar excess of RNA polymerase II was mixed with ^{32}P -labelled nucleosome cores. RNA polymerase II was prepared as described elsewhere³⁹, with modifications (P. Jat, R. I. Kamen, H. A. F. Mace and A. A. Travers, unpublished), without or with the final glycerol gradient step. Nucleosome cores were prepared from mouse myeloma NSO cells grown in culture. Nuclei were isolated from cells washed in 6 mM KCl, 1.5 mM NaCl, 1.5 mM Tris-HCl pH 7.4, 50 μM spermine, 15 μM spermidine, 1 mM MgCl_2 , 1 mM CaCl_2 , and lysed in the same buffer without MgCl_2 and CaCl_2 by centrifugation at 2,000 g then digested (2×10^8 nuclei ml^{-1}) with micrococcal nuclease (10 U ml^{-1}) in lysis buffer supplemented with 0.2 mM CaCl_2 and 0.2 mM MgCl_2 at 37 °C for 2 min. The digestion was stopped by adding EDTA to 1 mM, and chromatin solubilized by several resuspensions in a minimum volume of 0.2 mM EDTA pH 7.0 and centrifugations at 12,000g for 1 min. Nucleosome cores were prepared from chromatin as described²⁵, except on a much smaller scale, which allowed the concentration steps to be omitted and nucleosome cores to be isolated by sucrose gradient sedimentation (15–30.8% sucrose (w/w) isokinetic gradients⁴⁰ in 0.2 M NaCl, 10 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 0.2 mM phenylmethylsulphonyl fluoride, PMSF). Nucleosome cores (0.1 mg ml^{-1}) were labelled with ^{32}P at their 5' ends with polynucleotide kinase (20 U ml^{-1}) and [γ - ^{32}P]ATP in 50 mM Tris HCl pH 7.9, 2 mM dithiothreitol (DTT), 2 mM MgCl_2 , 5 μM ATP, 0.2 mM PMSF, purified from unincorporated [γ - ^{32}P]ATP by sucrose gradient sedimentation as above, dialysed against 10 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 0.2 mM PMSF, and used immediately for binding experiments. After supplementing with 5% glycerol (v/v), 0.2 mg ml^{-1} bovine serum albumin (BSA) and 2 mM DTT, nucleosome cores (2 μg ml^{-1}) were mixed with 0.05 vol. of 0.5 M Am_2SO_4 , 50 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 0.5 mM DTT, 25% glycerol containing RNA polymerase II and incubated on ice for 10 min, at 30 °C for 3 min, and again on ice for 10 min. Mixtures were layered onto SW60 15–30% (w/v) linear glycerol gradients containing 10 mM Tris-HCl pH 7.9, 2 mM DTT, 0.2 mM PMSF, 0.2 mg ml^{-1} BSA, centrifuged at 60,000 r.p.m. for 5 h, and the radioactivity in each fraction measured by scintillation counting. Sedimentation coefficients were measured in isokinetic sucrose gradients using nucleosome oligomers as markers. *b*, Various amounts of either unlabelled nucleosome cores or unlabelled DNA extracted from nucleosome cores (maximum final concentration 1 mg ml^{-1} in DNA) were added to a constant amount of labelled material, incubated with 0.06 mg ml^{-1} RNA polymerase II, the mixtures sedimented, and the fraction of radioactive nucleosome cores or of DNA bound to polymerase measured, all as described in *a*.

of procedures including sonication, nuclease digestion, salt precipitation and electrophoresis, sometimes resulting in chromatin fractions which are enriched in transcribed DNA sequences^{4–11}. While these results alone suggest that there must be something atypical about transcribed chromatin, it has proved more difficult to identify a particular protein component which might account for such a difference. A difference in the structure of active chromatin from that of total chromatin has been directly shown by its higher rate of digestion by DNase I¹². This increased susceptibility is lost when non-histone chromosomal proteins are removed, but can be restored by adding just two of the proteins, HMG-14 and HMG-17 (ref. 13). Further, HMG-14 and HMG-17 have been used as affinity ligands to isolate from chicken erythrocytes a nucleosome fraction enriched in the globin gene sequences¹¹. Other studies have associated the presence of these and other proteins, including ubiquitin-conjugated histone H2A¹⁴, acetylated histones^{15,16}

and RNA polymerase^{17,18}, with gene activity, and the presence of histone H1⁶ and a 50,000-molecular weight (M_r) protein called D1 with the lack of gene activity¹⁹.

These previously observed special properties of transcribed chromatin prompted us to ask whether eukaryotic RNA polymerase itself might specifically recognize some aspect of chromatin structure. Therefore, we have studied the interaction of RNA polymerase II with nucleosomes. For these experiments, we used nucleosome core particles, which contain only an octamer of two copies each of histones H2A, H2B, H3 and H4 wrapped by a defined length of 146 nucleotide pairs of DNA. As no histone H1 or non-histone chromosomal proteins are present in nucleosome cores, it was possible to study the role of just the four core histone proteins. The relative simplicity of nucleosome cores, together with a knowledge of their gross structure²⁰, has also allowed us to interpret their interaction with RNA polymerase II in detail.

Binding of RNA polymerase II to nucleosome cores

RNA polymerase II and nucleosome core particles were mixed at a 10-fold molar excess of polymerase in conditions where the polymerase was stable but, due to the absence of nucleotide triphosphates and divalent cations, not catalytically active. Nucleosome cores were labelled before mixing with polymerase with [γ - 32 P]ATP and polynucleotide kinase. The large size of the polymerase (M_r 600,000, 12–14 subunits, sedimentation coefficient 17S) compared to that of the nucleosome (M_r 190,000, 11S) allowed binding to be assayed simply by sedimentation. Figure 1a shows the sedimentation profile of a typical binding mixture: a portion of the nucleosomes (about 15%) sedimented at 18S, indicating that they were bound to polymerase. Analysis of the proteins in this peak by SDS-polyacrylamide gel electrophoresis shows that both polymerase proteins and histones are present, confirming that this is a polymerase–nucleosome core complex. A sedimentation coefficient of 18S is consistent with a complex containing one polymerase molecule and at least one nucleosome core particle. Most of the nucleosome cores sedimented at 11S, and were thus free of polymerase. Binding is faithful in that, when these unbound nucleosome cores were challenged again with polymerase, very little (<1%) binding was observed.

The binding profile in Fig. 1a shows that only a portion of nucleosome cores are bound to RNA polymerase II, even in the presence of a large excess of the polymerase. To investigate further the extent of nucleosome core binding, titrations were carried out at various polymerase/nucleosome core ratios. A titration experiment with 146-nucleotide pair DNA extracted from nucleosome cores was also carried out for comparison (Fig. 1b). The proportion of bound nucleosome cores reached a fairly level plateau at ~15%. This limited binding is in contrast to the situation where free DNA is titrated instead of nucleosome cores. In this case, DNA is simply bound in proportion to the amount added, up to the point where the DNA is saturated with polymerase. That essentially every polymerase molecule is competent to bind DNA is shown at low levels of added polymerase, where the polymerase is effectively saturated with DNA. Therefore, some nucleosome cores show a specific and selective binding to RNA polymerase II. As no selectivity in binding is seen for free DNA, this cannot directly be a property of the DNA sequence but rather of the nucleosome core particle.

Transcribed DNA sequences

As RNA polymerase II binds to a subset of nucleosome cores, it was natural to ask whether this subset was enriched in the DNA sequences transcribed by this enzyme. This was tested by hybridizing a cDNA probe made from total cytoplasmic poly(A)-containing mRNA to DNA extracted from total, unbound, and polymerase-bound nucleosome cores. The sequence complexity of such a mRNA preparation, from an exponentially growing mouse myeloma cell culture, has been analysed in detail²¹ and consists of highly abundant, moderately abundant, and rare sequence classes. The highly abundant fraction, comprised at least in part of immunoglobulin message, represents a significant proportion of the total mRNA, but is complementary to only an extremely small proportion of the genome and thus does not make a significant contribution in the hybridization analysis. The rare mRNA sequence population is complementary to a significant proportion of the genome, but each mRNA sequence is present in such small quantities that this fraction does not hybridize in our conditions and thus similarly does not make a significant contribution. Therefore, our hybridization assay detects transcribed sequences of moderate complexity, representing ~1,000 different genes with an average of ~100 RNA transcripts per cell.

The results of the hybridization experiment (Fig. 2) show that it requires less DNA from bound nucleosome cores and more DNA from unbound nucleosome cores to give the same

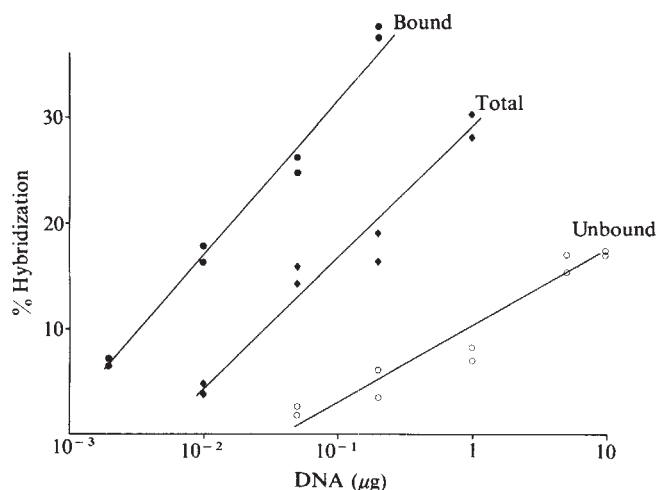


Fig. 2 Nucleosome cores bound to RNA polymerase II are heavily enriched in transcribed sequences. DNA from bound (●), unbound (○), or total (◆) nucleosome cores was hybridized to a cDNA probe synthesized from mouse myeloma cytoplasmic poly(A)-containing RNA²¹. Reaction mixtures (2 μ l) contained 50 μ M [α - 32 P]dATP (410 Ci mmol⁻¹), 50 μ M [α - 32 P]dCTP (410 Ci mmol⁻¹), 500 μ M dTTP, 500 μ M dGTP, 0.2 μ g oligo(dT), 1.0 μ g poly(A)-containing RNA, and 2 units of reverse transcriptase and reactions were carried out as described elsewhere⁴¹, resulting in cDNA transcripts ~600 nucleotides long. Hybridization mixtures (2 μ l) containing 0.4 μ g ml⁻¹ cDNA, 1–5 mg ml⁻¹ nucleosome core DNA, 2 mg ml⁻¹ *Escherichia coli* DNA, 1 M NaCl, 50 mM Tris-HCl pH 7.4, 0.1 mM EDTA were boiled and annealed at 67 °C for 48 h, representing a corrected R_{ot} of ~1 nucleotide-s per l. To detect the fraction of hybridized cDNA, samples were quickly added to 0.2 ml of 30 mM sodium acetate pH 4.5, 150 mM NaCl, 1 mM ZnCl₂, 0.1 mg ml⁻¹ tRNA, digested with 10 units of S₁ nuclease at 37 °C for 30 min, brought to 10% trichloroacetic acid (TCA), supplemented with 10 μ g tRNA carrier, placed on ice for 30 min, then precipitates were collected on Millipore filters, washed with 5% TCA and the radioactivity of the precipitates measured by scintillation counting. The exact total amount of cDNA in each sample was measured and the hybridization levels expressed as the percentage of that total, after subtracting background levels, which averaged 3% of the input radioactivity.

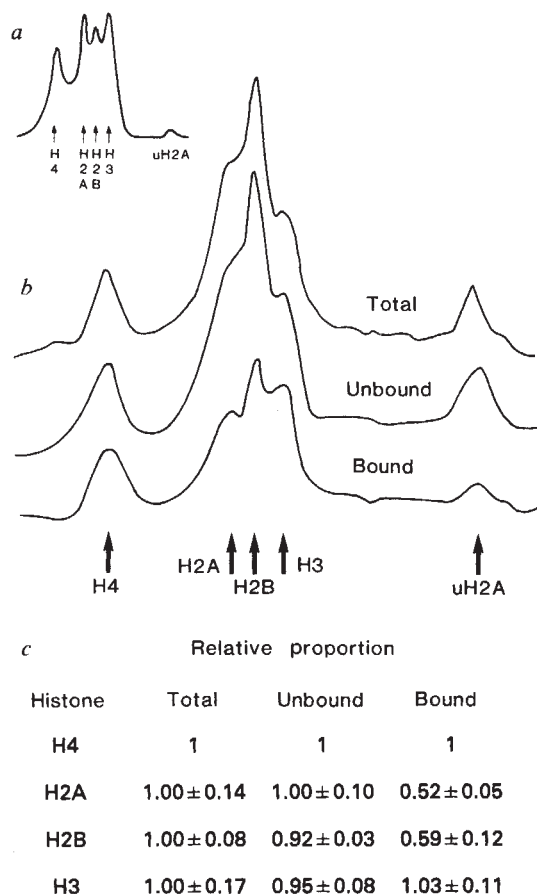
level of hybridization as DNA from total unfractionated nucleosome cores. The most direct way of making a quantitative comparison between the three DNA fractions is to note the amount of DNA required to give 10–20% hybridization, where the cDNA sequences in question are on average 3–5-fold in excess with respect to their complementary gene sequences and where the measured values are well above background. To achieve 15% cDNA hybridization, about 10 times as much total DNA as DNA from bound nucleosome cores is needed. Thus, DNA from bound nucleosome cores is about 10-fold enriched in these sequences—equal, within experimental error, to the maximum possible enrichment for a fraction representing one part in seven of the total nucleosome core preparation. About 80 times less total DNA than unbound DNA is needed to reach this level of hybridization, showing that the unbound fraction contains almost none (~1%) of these sequences. Therefore, essentially all the transcribed sequences are present in the nucleosome core fraction bound to the enzyme and consequently RNA polymerase II is binding selectively to nucleosome cores from transcribed genes.

Histone composition

The titration of RNA polymerase II with DNA extracted from nucleosome cores suggested that DNA sequence was not the factor responsible for selective binding. We therefore examined the histone composition of bound nucleosome cores and of total nucleosome cores to determine any differences which might account for the observed binding of polymerase to nucleosome cores from transcribed genes. To avoid confusion

Fig. 3 Nucleosome cores which bind to RNA polymerase II are deficient in histones H2A and H2B. *a*, Densitometer scan of Coomassie-stained gel of total nucleosome core preparation showing that the histones are present in about equimolar quantities, correcting for differences in molecular weight. uH2A refers to ubiquitin-H2A. *b*, Densitometer scans of ^{14}C -fluorograms of histones from total (top), unbound (middle) and RNA polymerase II-bound (bottom) nucleosome cores.

Methods: Nucleosome cores were prepared as described in Fig. 1 legend, from a cell culture that had been grown for 24 h with $5 \mu\text{Ci ml}^{-1}$ ^{14}C -arginine-free RPMI 1640 medium containing 5% fetal calf serum. Binding ($10 \mu\text{g}$ nucleosome cores per ml) and centrifugation were as described in Fig. 1a legend, except that an SW40 rotor was used at 40,000 r.p.m. for 14 h and the level of BSA in the gradient was reduced to $10 \mu\text{g ml}^{-1}$. Bound and unbound fractions, together with a portion of the total nucleosome core preparation, were precipitated with 5 mM spermine and $10 \mu\text{g ml}^{-1}$ added nucleosome carrier, precipitates collected by centrifugation at $15,000g$ for 15 min, dissolved in electrophoresis buffer, subjected to SDS-gel electrophoresis⁴² in a miniature apparatus without a stacking gel, and either stained with Coomassie blue or subjected to fluorography⁴³. RNA polymerase II was in some cases added to the unbound and total nucleosome cores before precipitation, with no measurable effect on the histones. Histones from total nucleosomes are labelled in approximate proportion to their lysine content, except for ubiquitin-H2A. *c*, Summary of results from three repetitions of the experiment described above. The proportions are corrected for differential labelling with lysine and are relative to each other, the level of histone H4 being set at unity in all cases. Ubiquitin-H2A was included in the H2A totals, taking into account its higher specific activity. Fluorograms from the three experiments were scanned in triplicate with a Joyce-Loebl scanning densitometer, the area under the curves measured, and the resulting values averaged for each entry.



with the small subunits of RNA polymerase II, nucleosome cores containing histones labelled with ^{14}C -lysine were used, resulting in a sucrose gradient profile indistinguishable from that of Fig. 1a. Samples were subjected to SDS and acid-Triton-urea gel electrophoresis, the histones were detected by fluorography, and densitometer profiles of the fluorograms were recorded (Fig. 3). Profiles from SDS gels of total, unbound and RNA polymerase II-bound nucleosomes are shown in Fig. 3b. The gel separates the four core histones and the ubiquitinated form of H2A^{22,23}. With the exception of ubiquitin-H2A (see below), the intensity of the histone bands in total nucleosomes is approximately proportional to their lysine content. Gels of the same nucleosome preparation stained with Coomassie blue (Fig. 3a) result in band intensities which are proportional to the histone molecule weights to within $\pm 10\%$, thus confirming that the four core histones are present in about equimolar quantities in the total nucleosome core preparation. Similarly, nucleosome cores which fail to bind to polymerase show equivalent molar ratios of the core histones. In contrast, nucleosome cores bound to RNA polymerase II show depleted levels of histones H2A and H2B—about half that of histones H3 and H4. Figure 3c summarizes the results of three such experiments. This difference suggests that only one copy each of histones H2A and H2B is present in nucleosome cores that bind RNA polymerase II. Acid-Triton-urea gel electrophoresis of the fractions revealed no consistent and significant differences in the various histone subspecies (not shown), except for ubiquitin-H2A.

The level of ubiquitin-H2A, as judged by fluorography, was consistently reduced by three-to-fivefold in nucleosome cores bound to polymerase. However, the level in labelled total nucleosome cores varied considerably in different experiments (from 10 to 30% of the total H2A) as did the mass fraction (from nearly undetectable levels up to 10%), judged by Coomassie blue staining. The higher specific activity of ubiquitin-H2A compared with that of the other core histone is apparently related to its higher turnover rate²⁴.

Structural analysis by nuclease protection

DNase I has been used previously to study quantitatively the accessibility of the DNA in nucleosome cores²⁵. This study provided a standard with which to compare the accessibility of the DNA in the RNA polymerase II-nucleosome core complex. The characteristic cutting pattern of DNase I on nucleosome cores is due to the histones restricting the availability of the DNA, such that DNase I initially makes cuts only at intervals of ~ 10 nucleotides from the DNA ends. Each site, from 1 to 13 along the 146-nucleotide pair DNA, has a characteristic frequency of digestion, which depends on how the local environment affects DNase I cutting. In practice, nucleosome cores labelled at their 5'-ends with ^{32}P are digested to several levels with DNase I and the resulting DNA fragments analysed by denaturing gel electrophoresis; the amount of radioactivity in a given band reflects the frequency of cutting by DNase I at the corresponding site in the nucleosome core. In our analysis, we compared this pattern with that obtained by digesting the RNA polymerase II-nucleosome core complex and measured how the accessibility had changed, thus allowing us to infer the type of structure which might be responsible.

Figure 4 shows the patterns obtained for the nucleosome cores that failed to bind to polymerase and for those of the RNA polymerase II-nucleosome core complex. The digestion pattern of the complex differs from that of the unbound nucleosome cores in two respects. First, the cutting is distributed more evenly along the whole length of the DNA, reflected both in less variation in cutting from site to site and in much greater cutting between sites; this indicates a type of large-scale opening of the structure on RNA polymerase II binding. Second, the digestion pattern can be interpreted site by site. In the complex, site one (S1; ~ 10 nucleotides from the ^{32}P -labelled termini), S2, S4, S5 and S13 are all protected, while S3, S6, S7 and S8 are exposed. These represent absolute changes in the rate of digestion at each site for a given level of DNase I, not merely relative changes from one site to another. The overall rates of

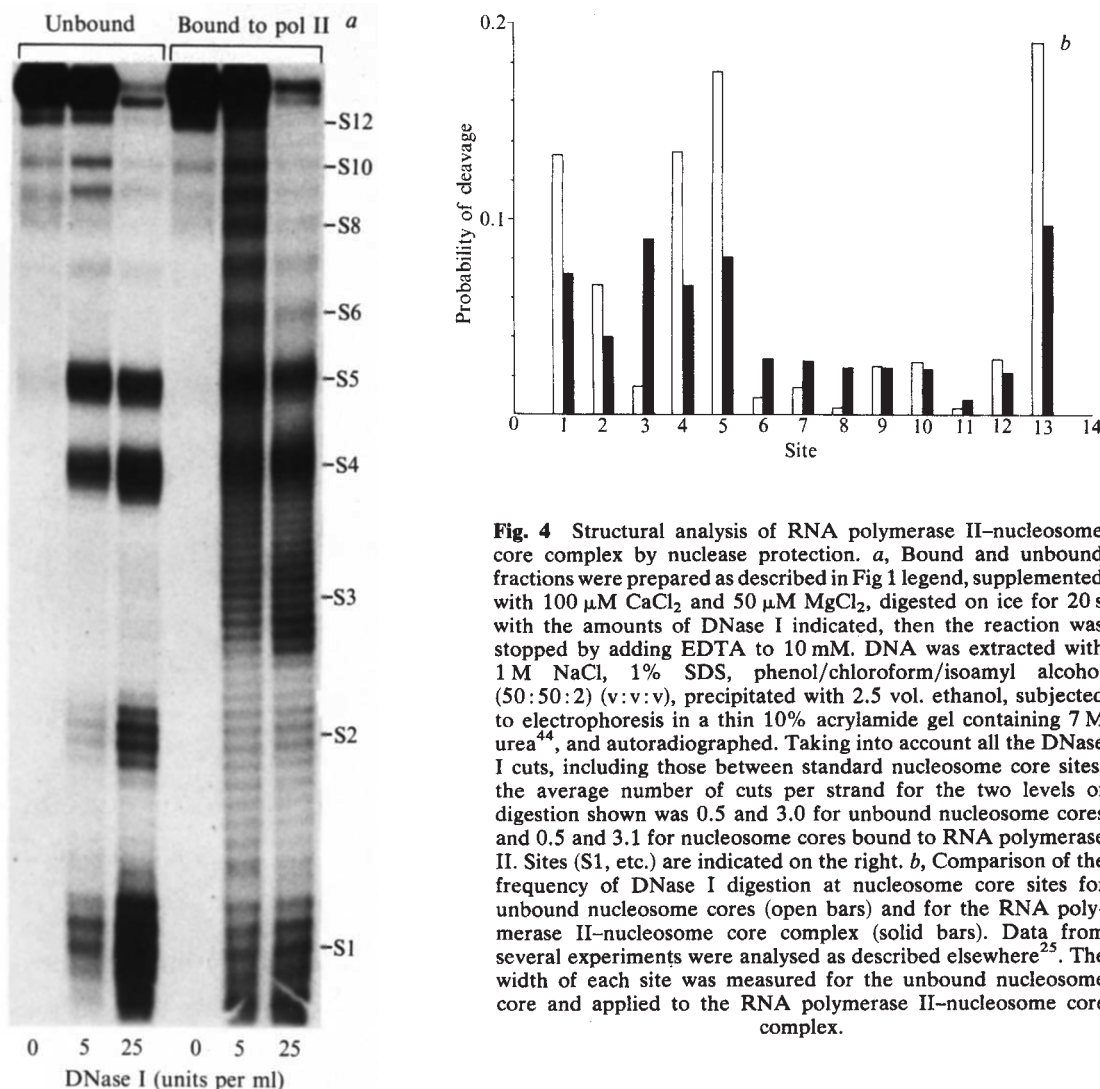


Fig. 4 Structural analysis of RNA polymerase II-nucleosome core complex by nuclease protection. *a*, Bound and unbound fractions were prepared as described in Fig 1 legend, supplemented with 100 μ M CaCl_2 and 50 μ M MgCl_2 , digested on ice for 20 s with the amounts of DNase I indicated, then the reaction was stopped by adding EDTA to 10 mM. DNA was extracted with 1 M NaCl, 1% SDS, phenol/chloroform/isoamyl alcohol (50:50:2) (v:v:v), precipitated with 2.5 vol. ethanol, subjected to electrophoresis in a thin 10% acrylamide gel containing 7 M urea⁴⁴, and autoradiographed. Taking into account all the DNase I cuts, including those between standard nucleosome core sites, the average number of cuts per strand for the two levels of digestion shown was 0.5 and 3.0 for unbound nucleosome cores and 0.5 and 3.1 for nucleosome cores bound to RNA polymerase II. Sites (S1, etc.) are indicated on the right. *b*, Comparison of the frequency of DNase I digestion at nucleosome core sites for unbound nucleosome cores (open bars) and for the RNA polymerase II-nucleosome core complex (solid bars). Data from several experiments were analysed as described elsewhere²⁵. The width of each site was measured for the unbound nucleosome core and applied to the RNA polymerase II-nucleosome core complex.

digestion (that is, disappearance of radioactivity from the starting 146-nucleotide DNA band) for unbound nucleosome core and for the RNA polymerase II-nucleosome core complex are very similar, reflecting the approximate cancellation of protection effects, enhancement effects and increased digestion between standard nucleosome core cutting sites.

To exclude the possibility that RNA polymerase II binds to the ends of the DNA, the accessibility of the 5'-termini was probed by digestion with bacterial alkaline phosphatase (M, 80,000). ^{32}P was released from the complex at an even higher rate (~50% higher) than from unbound nucleosome cores (not shown). Thus, RNA polymerase II does not protect the DNA ends from phosphatase and does not seem to bind in this vicinity.

When the RNA polymerase II-core complex was treated with 0.3 M KCl, the polymerase was released, resulting in a nucleosome core having a sedimentation coefficient of ~11S (Fig. 5a). DNase I digestion of the released nucleosome cores revealed a pattern of fragments similar to that of bulk nucleosome cores and very different from that of the RNA polymerase II-nucleosome core complex (Fig. 5b). Thus, the original DNase I protection pattern is largely restored on release of polymerase, showing that the change in structure afforded by RNA polymerase II is for the most part reversible. However, quantitative analysis shows that the overall rate of digestion of nucleosome cores released from polymerase is 1.6 times greater than that of the control nucleosome core preparation. Site-by-site analysis (not shown, but as in Fig. 4b) shows that most of this increase in digestion rate is concentrated at S1 (2.1 times), S2 (3.1 times) and S13 (1.4 times). The increase in the

accessibility of the DNA to DNase I at these sites can be correlated with the reduced levels of H2A and H2B in the nucleosome cores bound to RNA polymerase II (see below).

Relationship to other work

The results presented here show a specific interaction between RNA polymerase II and nucleosome cores from genes encoding mRNA. These nucleosome cores are deficient in histones H2A and H2B and this deficiency may be responsible for their specific interaction with RNA polymerase II. Transcription studies *in vitro* using a chromatin template (reconstituted from DNA and the four core histones) and purified RNA polymerase II have shown a greatly reduced level of transcription compared with that of naked DNA²⁶. This inhibition was shown to be due to a decreased rate of transcript elongation and could be relieved by increasing the ionic strength. Inhibition was relieved completely at 0.35 M Am_2SO_4 , in which conditions²⁷ (or in corresponding levels of NaCl^{28,29}) H2A and H2B are dissociated while H3 and H4 remain bound to the DNA. Reduced levels of H2A and H2B might explain the stimulation of transcription in those studies and the selective binding reported here.

A study of the mononucleosomes released at early stages of micrococcal nuclease digestions of trout testis nuclei³⁰ has shown that these nucleosomes are about 40% pure in cytoplasmic poly(A)-containing mRNA sequences. A subfraction of these mononucleosomes contains levels of H2A and H2B which are about half those of H3 and H4. For the enriched fraction as a whole, we calculate from their data that the amount of H2A plus H2B is ~70% that of H3 plus H4. Allowing for the fact that this fraction is only 40% pure in mRNA sequences,

Fig. 5 Disassociation of RNA polymerase II from nucleosome cores by salt treatment. The RNA polymerase II–nucleosome core complex (Fig. 1) was dialysed against 0.3 M KCl, 10 mM Tris-HCl pH 7.9, 10 mM Am_2SO_4 , 2 mM DTT, 0.2 mM PMSF for 4 h and recentrifuged (a) in a glycerol gradient, as described in Fig. 1 legend. A control nucleosome core sample, which had not been subjected to RNA polymerase II binding, was also dialysed against this buffer and centrifuged. (When the complex was dialysed against the above buffer without 0.3 M KCl and recentrifuged, most of the radioactivity still sedimented at 18S). The released nucleosome cores and the control nucleosome cores were digested with DNase I and DNA fragments analysed by gel electrophoresis (b) as described in Fig. 4 legend. The average number of DNase I cuts per strand was 0.4, 0.6, 1.0 and 2.2 for the control nucleosome core preparations and 0.7, 0.9, 2.1 and 3.1 for the nucleosome cores released from RNA polymerase.

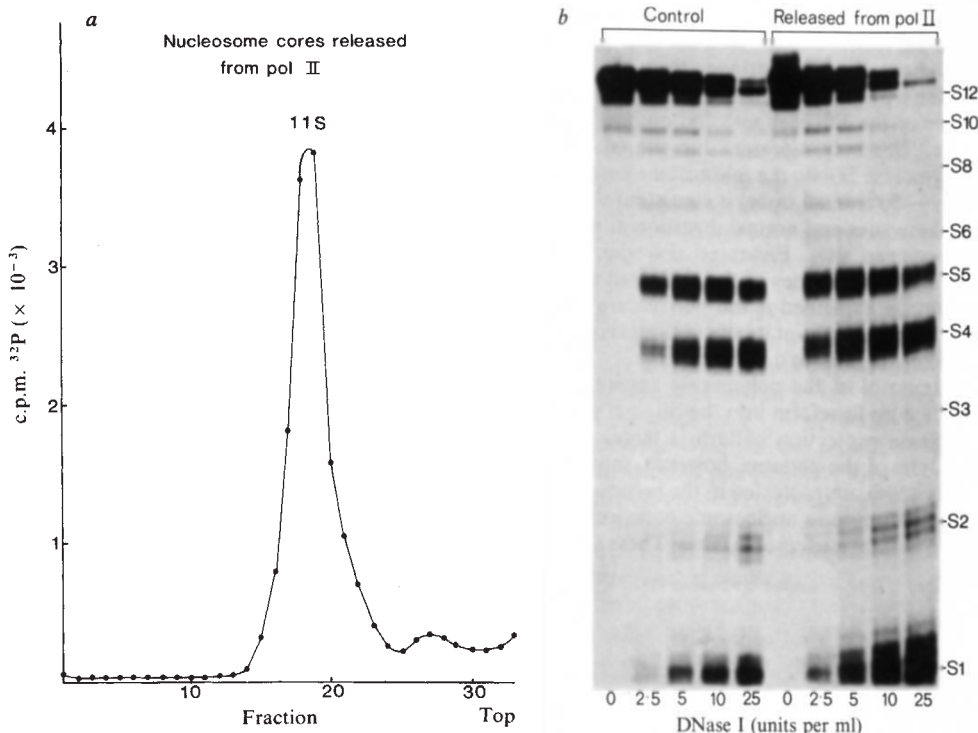
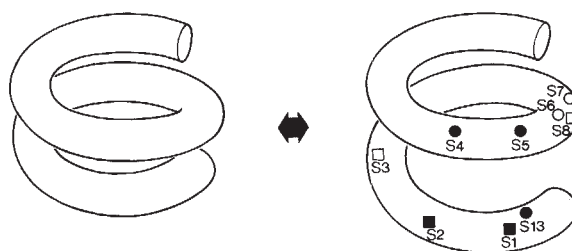


Fig. 6 Interpretation of the nuclease protection data. A model of the nucleosome core DNA (left) and the changes associated with RNA polymerase II binding (right). The symbols represent decreased (solid) or increased (open) digestion by DNase I at sites in the complex (Fig. 4b). Only one of each pair of DNase I sites arising from both DNA strands is indicated, with site 1 (S1), S2, S3 and S8 on one strand (squares) and S4, S5, S6, S7 and S13 on the other strand (circles). Dissociation of RNA polymerase II largely restores the usual DNase I digestion pattern, but some differences remain, which can be attributed to the decreased levels of histones H2A and H2B in these nucleosome cores.



this is in good agreement with the stoichiometry of the four core histones which we observe in nucleosome cores bound to RNA polymerase II.

The reduced levels of H2A and H2B, to about one-half that of a full core octamer, are consistent with the internal organization of the histone octamer²⁰ (one H3–H4 tetramer flanked by two H2A–H2B dimers) and suggest the dissociation of one of the two H2A–H2B dimers. We propose that the absence of an H2A–H2B dimer is related to the transcription of genes in chromatin. The proportion of nucleosomes that bind to RNA polymerase II (15%) suggests that polymerase can recognize nucleosome cores from a large portion of a gene. This is in accord with the fact that purified RNA polymerase II does not initiate transcription at *in vivo* promoter sites³¹ and implies that the interaction observed is related to elongation, perhaps representing a pause in transcription through the nucleosome.

We believe that chromatin, containing transcribed genes, may be deficient in H2A and H2B in the cell, but we have no way of knowing how H2A and H2B in these nucleosomes might be selectively removed, nor can we exclude the possibility that H2A and H2B were lost during the experimental procedure. If H2A and H2B simply dissociate or are proteolysed at some stage after cell disruption, they do so in relationship to the subset of nucleosomes that are transcribed by RNA polymerase II in the cell. Absence or removal might be related to a modified histone or histone subspecies that is not detected by acid–Triton–urea gel electrophoresis, to a combination of different histone modifications, or to ubiquitination. H2B has been found in a ubiquitinated form³², although at a much lower level than H2A. Ubiquitination can increase the rate of hydrolysis by a specific ATP-dependent proteolytic system of the protein to which it is conjugated³³. Another possibility is that H2A and H2B are removed during incubation with polymerase. While

we have not observed free histones (or histone fragments) in the gradient profiles of the sedimented mixtures, they may have gone undetected.

Other studies on transcribed fractions of chromatin have found widely varying ratios of the four core histones³. A recent study of nucleosomes fractionated by HMG-14 and HMG-17 affinity chromatography¹¹ indicated that the active nucleosomes contain equimolar quantities of the core histones. However, the same study showed that added H2A and H2B, but not H3 and H4, compete for HMG-14 and HMG-17 binding and prevent HMG-14 and HMG-17 from restoring DNase I sensitivity to nucleosomes from transcribed genes³⁴.

The results from another study of the binding of RNA polymerase II (from wheat germ) to nucleosomes³⁵ differ from those reported here, primarily in that selective binding was not observed. This unrestricted binding was probably due to damage in the nucleosome preparations, documented in that same study, although we have not observed the concentration-dependent dissociation of nucleosomes that they report.

Model of RNA polymerase II–nucleosome core complex

The nuclease protection data of nucleosome cores bound to polymerase show, in addition to an apparent opening of the nucleosome core structure, that certain sites are protected while others are exposed. These changes are for the most part reversible on dissociation of the polymerase. Figure 6 shows an interpretation of these results. Nucleosome cores have an overall 2-fold axis of symmetry²⁰ and therefore DNase I sites are likely to exist in equivalent pairs²⁵. The RNA polymerase II–nucleosome core complex may not be symmetrical and in Fig. 6 we specifically interpret the data in terms of an asymmetrical complex, with one of two potential binding regions

occupied by polymerase. If protection at S1 and S2 is assumed to arise from one DNA strand and protection at S4, S5 and S13 from the other strand, then all five protected sites fall within a patch, 10–15 nucleotide pairs long and straddling both turns of the DNA superhelix, on one side of the nucleosome core. This may represent the site of direct contact of RNA polymerase II with the nucleosome core. The degree of protection (~50% in all cases) is consistent with complete protection at these sites and normal digestion at the corresponding set of unoccupied sites. Enhanced digestion at S3, S6, S7 and S8 occurs on both sides of the protected region (see Fig. 6). This might be explained if the polymerase forces apart the DNA superhelical turns at its site of direct contact, thus exposing the flanking regions to digestion.

Removal of the polymerase apparently allows the nucleosome core to reform into the original structure, as the original nuclease protection pattern is largely restored. Quantitative analysis of the patterns, however, shows that S1, S2 and S13, sites which are protected in the presence of polymerase, are all digested more in nucleosome cores released from polymerase than in total nucleosome cores. These sites correspond with the

location of histones H2A and H2B at the end regions of nucleosome core DNA^{20,36} and we therefore believe that increased digestion at these sites is due to the deficiency of histones H2A and H2B in nucleosome cores released from RNA polymerase II.

Of course, non-histone proteins and histone H1 may also be involved in polymerase binding and experiments are in progress to determine their role, if any. For example, HMG-14 and HMG-17 bind to the S1 and S2 region of nucleosome core DNA^{37,38} and it could be that their function is to "hold the place" for RNA polymerase II in the absence of H2A and H2B. In addition, it seems unlikely that the entire RNA polymerase II molecule is directly involved in nucleosome core binding and it should now be possible to determine which subunit (or subunits) is responsible.

We thank M. Searles for technical assistance; A. Travers and H. Mace for help with RNA polymerase II preparations; and A. Klug and A. Travers for criticism of the manuscript. B.W.B. was supported by an EMBO Long Term Fellowship and by a Damon Runyon-Walter Winchell Postdoctoral Fellowship Grant.

Received 22 October; accepted 29 November 1982.

1. Kornberg, R. D. A. *Rev. Biochem.* **46**, 931–954 (1977).
2. Lacy, E. & Axel, R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3978–3982 (1975).
3. Mathis, D., Oudet, P. & Chambon, P. *Proc. Nucleic Acids Res.* **24**, 1–55 (1980).
4. Frenster, J. H., Allfrey, Y. G. & Mirsky, A. E. *Proc. natn. Acad. Sci. U.S.A.* **50**, 1026–1032 (1963).
5. Marushige, K. & Bonner, J. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2941–2944 (1971).
6. Gottesfeld, J. M., Garrard, W. T., Bagl, G., Wilson, R. F. & Bonner, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2193–2197 (1974).
7. Berkowitz, E. M. & Doty, P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3328–3332 (1975).
8. Bloom, K. S. & Anderson, J. N. *Cell* **15**, 141–150 (1978).
9. Levy-Wilson, B. & Dixon, G. H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1682–1686 (1979).
10. Levinger, L., Barsoum, J. & Varshavsky, A. *J. molec. Biol.* **146**, 287–304 (1981).
11. Weisbrod, S. & Weintraub, H. *Cell* **23**, 391–400 (1981).
12. Weintraub, H. & Groudine, M. *Science* **193**, 848–856 (1976).
13. Weisbrod, S. & Weintraub, H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 630–634 (1979).
14. Levinger, L. & Varshavsky, A. *Cell* **28**, 375–385 (1982).
15. Vidall, G., Botta, L. C., Bradbury, E. M. & Allfrey, V. G. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2239–2243 (1978).
16. Nelson, D., Covault, J. & Chalkley, R. *Nucleic Acids Res.* **8**, 1745–1763 (1980).
17. Tata, J. R. & Baker, B. J. *J. molec. Biol.* **118**, 249–272 (1978).
18. Hataiyama, T., Omori, K., Inoue, A. & Yukioka, M. *Biochim. biophys. Acta* **652**, 245–255 (1981).
19. Levinger, L. & Varshavsky, A. *Cell* **28**, 375–385 (1982).
20. Klug, A., Rhodes, D., Smith, J., Finch, J. T. & Thomas, J. O. *Nature* **287**, 509–515 (1980).
21. Mechler, B. & Rabbitts, T. H. *J. Cell Biol.* **88**, 29–36 (1981).
22. Goldknopf, I. L. & Busch, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 864–868 (1977).

23. Goldknopf, I. L., French, M. F., Musso, R. & Busch, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5492–5495 (1977).
24. Seale, R. L. *Nucleic Acids Res.* **9**, 3151–3158 (1981).
25. Lutter, L. C. *J. molec. Biol.* **124**, 391–420 (1978).
26. Wasyluk, B. & Chambon, P. *Eur. J. Biochem.* **98**, 318–327 (1979).
27. Spelsberg, T. C. & Hnilica, L. S. *Biochim. biophys. Acta* **228**, 202–211 (1971).
28. Ohlenbusch, H. H., Olivera, B. M., Tuan, D. & Davidson, N. *J. molec. Biol.* **25**, 299–315 (1967).
29. Stockley, P. G. & Thomas, J. O. *FEBS Lett.* **99**, 129–135 (1979).
30. Hutcheon, T., Dixon, G. H. & Levy-Wilson, B. *J. biol. Chem.* **255**, 681–685 (1980).
31. Roeder, R. G. in *RNA Polymerase* (eds Losick, R. & Chamberlin, M.) 285–330 (Cold Spring Harbor Laboratory, New York, 1976).
32. Wu, R. S., Kohn, K. W. & Bonner, W. M. *J. biol. Chem.* **256**, 5916–5920 (1981).
33. Ciechanover, A., Heller, H., Katz-Etzion, R. & Herskha, A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 761–765 (1981).
34. Weisbrod, S., Groudine, M. & Weintraub, H. *Cell* **19**, 289–301 (1980).
35. Lilley, D. M. J., Jacobs, M. F. & Houghton, M. *Nucleic Acids Res.* **7**, 377–399 (1979).
36. Mirzabekov, A. D., Shick, V. V., Belyavsky, A. V. & Bavykin, S. G. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4184–4188 (1978).
37. Mardian, J. K. W., Paton, A. E., Bumick, G. J. & Olins, D. E. *Science* **209**, 1334–1336 (1980).
38. Sandeen, G., Wood, W. I. & Felsenfeld, G. *Nucleic Acids Res.* **8**, 3757–3778 (1980).
39. Hodo, H. G. & Blatti, S. P. *Biochemistry* **16**, 2334–2343 (1977).
40. McCarty, K. S. Jr, Vollmer, R. T. & McCarty, K. S. *Analyt. Biochem.* **61**, 165–183 (1974).
41. Buell, G. N., Wickens, M. P., Payvar, F. & Schimke, R. T. *J. biol. Chem.* **253**, 2471–2482 (1978).
42. Thomas, J. O. & Kornberg, R. D. *Meth. Cell Biol.* **18**, 429–440 (1978).
43. Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335–341 (1975).
44. Sanger, F. & Coulson, A. R. *FEBS Lett.* **87**, 107–110 (1978).

LETTERS TO NATURE

A radio galaxy at high redshift undergoing strong stellar evolution

S. J. Lilly, M. S. Longair & I. S. McLean

Department of Astronomy, University of Edinburgh, and Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ, UK

Evidence has been accumulating^{1–4} for strong evolutionary effects in the stellar populations of giant elliptical galaxies with cosmological epoch. In several studies of elliptical galaxies at high redshifts, including our own investigation into the colours of 3C radio galaxies, it has been apparent that the galaxies are generally considerably bluer at earlier epochs. Using a CCD imaging photometer we have now investigated the location of the excess of short wavelength light in one 3C radio galaxy, 3C352, at a redshift of 0.81. The excess is present throughout the galaxy and is not concentrated at the nucleus. This supports the hypothesis that it is caused by an evolving stellar population and indicates that the regions of star formation are not concentrated at the nucleus.

Early work by Kristian *et al.*¹ on the colours of giant elliptical galaxies has been followed by studies involving galaxies at higher redshifts: Gunn *et al.*² have found changes in the optical spectra and (*g-r*) colours of first ranked cluster ellipticals at

redshifts >0.4, compared with nearby galaxies of the same type; Van der Laan and Windhorst³ have shown colour changes in a large sample of faint radio galaxies which they infer to have redshifts between 0.5 and 1.0; we⁴ have recently presented evidence that the optical-IR colours (*V-K*) and (*R-K*) of giant elliptical galaxies associated with powerful 3C radio sources with measured redshifts of around 1 are markedly different from those expected on the basis of observations of 3C galaxies at lower redshifts. All these changes are in the sense of an increase in the shorter wavelength fluxes of these galaxies, and are usually interpreted as being caused by an increase in the number of massive young stars.

The nature of the evolutionary changes found in 3C radio galaxies is shown in Fig. 1. We have used our previously published IR data⁴ and some more recent unpublished data to construct (*V-K*) colours for a sample of radio galaxies spanning a large redshift range; the sources of the optical photometry are given in ref. 4. We have also converted some of the *r* magnitudes ($\lambda_c = 6,550$) to effective *V* magnitudes by assuming *V-r* = 1.0 and 0.8 for *z* = 0.7 and 1.0 respectively. This transformation is appropriate to the evolving models which fit the (*R-K*) and (*V-K*) colours of the high redshift radio galaxies, and is used to show that all the radio galaxies may be represented by a single relation in an optical-IR colour redshift diagram. We have plotted on Fig. 1 only those radio galaxies whose

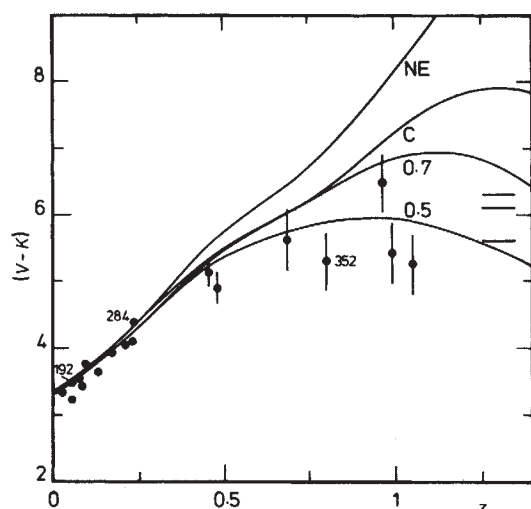


Fig. 1 The changes in optical-IR colours of 3C radio galaxies with redshift. The lines indicate various predictions for evolving stellar populations from ref. 7, which are described in the text. Some of the high redshift V magnitudes have been derived from CCD r magnitudes, IR data are from our previous published⁴ and unpublished work. Galaxies with unknown z are shown as horizontal bars of arbitrary length. The errors on their colours are the same as for the other high z galaxies.

Table 1 Flux densities and observed magnitudes of 3C352

	λ_c (Å)	FWHM (Å)	Mag	Flux (μ Jy)
B'	4,647	730	22.56 ± 0.2	4.12 ± 0.9
V'	5,266	937	22.59 ± 0.2	3.66 ± 0.8
R'	7,591	772	20.69 ± 0.15	13.9 ± 2.5
I'	8,488	950	19.63 ± 0.2	33 ± 10
K	22,000	4,800	17.24 ± 0.12	82 ± 10

Calibration of the B' , V' , R' and I' magnitudes was based on observations of five standard stars, and this led to uncertainties in the calibration of up to 10% for B' , V' and R' and <20% for I' . B and V magnitudes of the bright star derived from our B' and V' measurements agree to about 0.05 mag with those published by Kristian *et al.*⁸. Also our V' value for 3C352 is similar to the V_{scan} magnitude of Smith *et al.*⁹ when allowance is made for the much larger aperture used in the present investigation. A smaller aperture of 3 arc s diameter has $V' = 23.0$ compared with $V_{\text{scan}} = 23.2$.

colours are not seriously contaminated by non-stellar radiation associated with the nucleus of the radio galaxy. The elimination of other 3C galaxies was based on three criteria. First, we have included only those sources with Fanaroff-Riley Class II radio structure, that is the classical double radio sources. Second, we have excluded those galaxies that are known to have broad emission lines (BLRG). Our previous work has shown⁶ that the low redshift BLRGs in our sample had optical-IR continua dominated by non-stellar radiation components. Finally, we have excluded galaxies which have a prominent unresolved nucleus on deep images since this indicates that the emission is dominated by either a non-stellar continuum or by a strong emission line, both of which would result in misleading colours. In addition, there are two further arguments that give us increased confidence that the colours of the galaxies remaining in the sample and in particular the faint galaxies at $z > 0.5$, are not seriously affected by a non-stellar nuclear component. First, several of these galaxies show stellar absorption features in their spectra which would be easily washed out by the addition of a featureless non-stellar continuum. Second, Hine and Longair⁶ have demonstrated that radio galaxies with the most optically active nuclei have strong central radio components relative to the total radio flux density, whereas radio galaxies which have either a pure stellar spectrum or a narrow emission line spectrum have very weak central radio components. All the galaxies in Fig. 1 with $z > 0.5$, and in particular those without

measured redshift, and hence without spectroscopic information, have central radio component flux densities which are a very small fraction of the total and in many cases no central component has been detected at all. This suggests that these galaxies do not have optically active nuclei.

The observed optical-IR colours may be compared as a function of redshift with the predictions of various models of an evolving stellar population constructed by Bruzual⁷ and illustrated in Fig. 1. In brief, these models assume a universal initial mass function and differ principally in having different star formation rate (SFR) histories. The three evolutionary models plotted represent the star formation rate by a burst of star formation of 1,000 Myr duration, (the C-model) and by two exponentially decaying SFRs ($\mu = 0.7$ and $\mu = 0.5$, the parameter μ indicating the mass fraction of the galaxy in the form of stars at the end of the first 1,000 Myr). The no-evolution (NE) model has an unchanging spectral energy distribution at all epochs. For the reasons discussed above we have interpreted our results as being caused by changes in the stellar populations of these galaxies. If this is correct, then models without some continuing star formation in the range $0.5 < z < 1$ do not fit the available data well. Some very young stars are required to produce the amount of short wavelength light observed. Note that the same evolving models can account for the data of Van der Laan and Windhorst¹. However, a test of these results and of their interpretation is to study the spectral energy distribution at a wider range of wavelengths and in particular it is of considerable interest to use an imaging photometer to determine the location of the source of emission of the excess of short wavelength light. A few 3C radio galaxies in our sample at low redshift have non-stellar UV components in their spectra (for example, 3C33), associated with the active nucleus. If the blue excess that we observe in the $(V-K)$ diagram at high redshift was found to be emitted from the nuclear region then it would be possible that the excess was caused by a similar non-stellar component, especially as the high redshift 3C radio galaxies must be intrinsically more powerful in the radio compared with nearby 3C galaxies.

We have been able to carry out this investigation for 3C352: a typical high redshift 3C galaxy. It was identified by Kristian *et al.*⁸ and has a secure redshift of 0.806 based on five narrow emission lines⁹. In addition the 4,000 Å stellar absorption feature is probably present in the spectrum⁹. The galaxy is symmetrically placed between the components of the double radio source which is 10 arc s in size, and a central radio component has not been detected.

The Royal Observatory Edinburgh Imaging Spectropolarimeter⁵ consists of a photometer with CCD readout that can be operated in various modes including that of direct imaging. Four exposures of 3C352 were taken during the commissioning of the instrument on the 3.8-m United Kingdom Infrared Telescope in April 1982. The exposures were taken through passbands, defined by interference filters, that correspond roughly to B , V , R and I . They are not identical to the standard wavebands, however, and will be referred to as B' , V' , R' and I' . The images were reduced at Edinburgh on the Starlink network using in part the E2D package. The four images are shown in Fig. 2. The seeing in each exposure was ~ 1.5 arc s. 3C352 is the faint image situated between the radio lobes indicated by white crosses.

The images of the galaxy are visible on all four exposures. Furthermore, the B' and V' images are extended and elongated. They are clearly not dominated by the nucleus of the galaxy. Indeed comparison of the surface brightness distribution shows that the B' and V' images are less centrally concentrated than the R' and I' . The most compact image is at I' , in which the galactic image is barely resolved. 3C352 has very strong $\lambda 3,727$ [O II] emission^{9,10}, having an equivalent width of 540 Å, and it is therefore natural to attribute the appearance of the I' image to the presence of the redshifted $\lambda 5,007$ [O III] line emitted from close to the nucleus in the red wing of the I' filter. On detailed examination, it is apparent that the galactic images

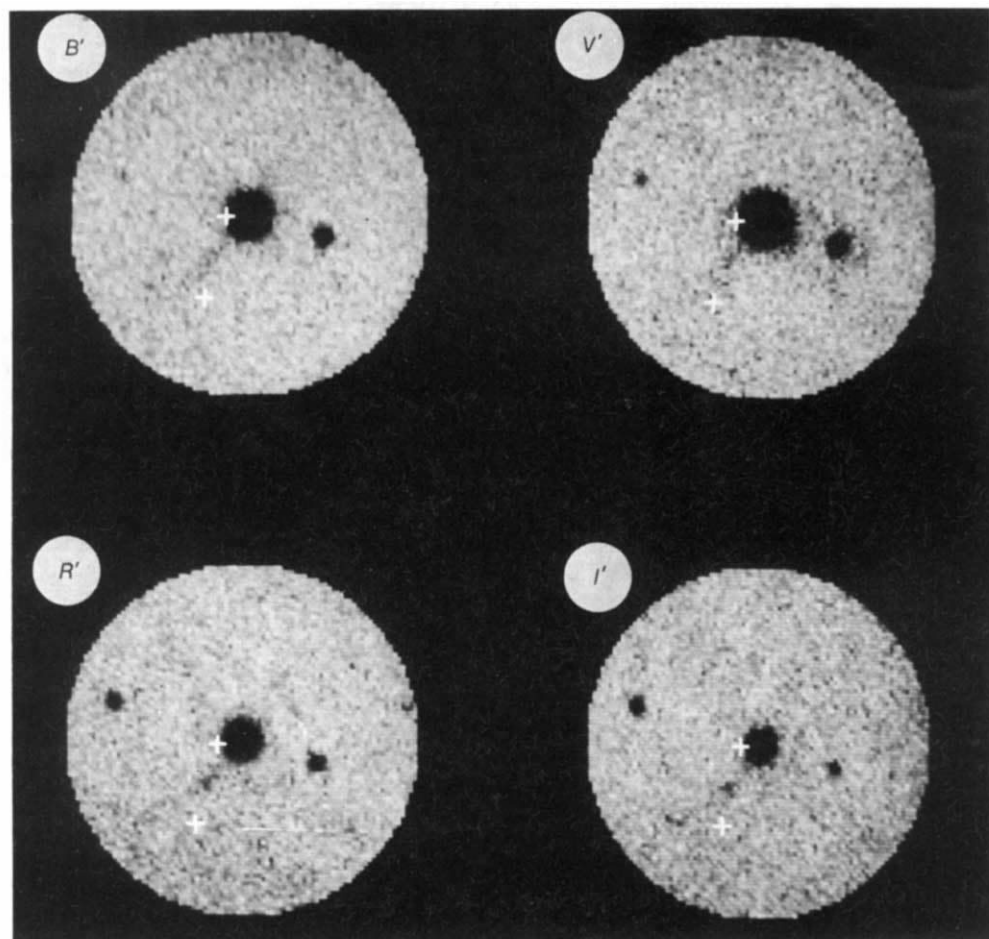


Fig. 2 Images in four colours of 3C352. The compactness of the I' image is probably caused by strong $\lambda 5,007 \text{ \AA}$ [O III]. The extended nature of the B' and V' images indicates that the excess of short wavelength flux density does not originate from the nucleus of the galaxy. The radio lobes are separated by 10 arc s.

in B' and V' are extended along the axis of the galaxy to a greater distance from the star than is evident on our R' and I' images or on the deep red photograph of Smith *et al.*⁹. The nature of this extension is not clear—it may represent the outer regions of the galaxy which were brighter in the past or it might be due to a rather blue companion galaxy.

We have therefore measured the flux density in all wavebands through the same aperture located in exactly the same position relative to the bright star. This aperture was rectangular 5×7 arc s and was centred on the maxima of all four images, which were coincident. The major axis of the aperture was parallel to that of the galaxy, and covered the extent of the R' and I' images, and hence did not include the extension described above.

It was also similar in size to the circular 7.2 arc s aperture used in our IR K measurement. The flux densities and observed magnitudes (based on α -Lyrae) are given in Table 1, together with details of the passbands used. The internal uncertainties in the flux density measurements on each image were $<20\%$, being caused by photon noise and uncertainties in determining the local sky value.

In Fig. 3, these flux density measurements, together with our earlier⁴ K ($2.2 \mu\text{m}$) measurement, are shown compared with the rest frame spectral energy distribution of a 3C radio galaxy at much smaller redshift, 3C192 ($z = 0.06$). This too is a classical double radio source with a narrow emission line spectrum. The optical spectrophotometry is by Yee and Oke¹¹ and the IR flux densities are from our earlier work⁴. In addition we show the broad band B' , V' , R' , I' , J , H and K flux densities of 3C284, a radio galaxy at intermediate redshift ($z = 0.239$), which were obtained during the same observing run and reduced in an identical fashion to those of 3C352. The good agreement of the broad band colours of 3C284 with the SED of 3C192 gives us confidence that our calibration of the four broad band colours is accurate.

Figure 3 shows that the flux density of 3C352 from that part of the spectrum shortward of $4,000 \text{ \AA}$ is substantially enhanced. As a crude indication of the magnitude of the effect, the addition of some 10^4 05 stars is required to match the observed flux density in the rest frame U waveband. Another comparison is that the $B' - K$ colour is about that expected¹² for a typical Sab or Sbc galaxy at $z = 0.81$. We believe, however, that we are observing an elliptical galaxy as the double radio structure of 3C352 is never associated at low redshifts with spiral galaxies. We interpret the large I' flux density as being caused by the $\lambda 5,007$ [O III] line that also produces the peaked appearance of that image. The strength of the $\lambda 3,727$ [O II] line was measured by Smith *et al.*⁹ to be $0.6 \times 10^{-17} \text{ W m}^{-2}$. As we would expect $\lambda 5,007$ [O III] to be stronger than this, this line may account for a considerable fraction of the observed flux of $\sim 1.3 \times 10^{-17} \text{ W m}^{-2}$ in the I' passband, even though the redshifted line is in the red wing of the filter, where the relative transmission is $\sim 35\%$. The appearances of the other images, and a study of the spectrum and line strengths of 3C352 (ref. 9) make it unlikely that the other wavebands are seriously affected, both $\lambda 3,869$ [Ne III] and the very strong $\lambda 3,727$ [O II] being outside the passbands used.

The excess of short wavelength light appears to be distributed throughout the region of the galaxy visible on the R' exposure and the deep R plate of Smith *et al.*⁸ and is definitely not concentrated at the centre of the galaxy. The difference between the appearance of the I' image, which we have argued is dominated by nuclear emission and that of the B' image is obvious. This result supports the hypothesis that the changes are being caused by the stellar population and not by a nuclear non-stellar component. Furthermore, note that there appears to be considerable star formation in the outer regions of the galaxy away from the nucleus. This result must be treated with some caution, but if confirmed in larger samples of galaxies, will be important for theories of galactic evolution. We suspect

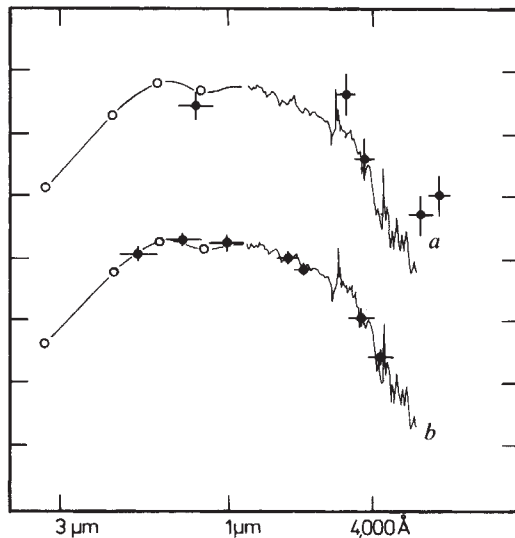


Fig. 3 The broadband rest-frame spectral energy distributions of 3C352 compared with 3C192 ($z = 0.060$) and 3C284 ($z = 0.239$). *a*, The optical spectrophotometry of 3C192 of ref. 11, with the broadband fluxes of 3C352 superimposed. *b*, The broadband fluxes of 3C284 superimposed on the scan of 3C192. All IR data are from Lilly and Longair⁴. The vertical scale is in units of $2.5 \log \nu f_\nu$.

though that other high redshift radio galaxies will show similar effects first because 3C352 does not have atypical colours compared with the other high redshift galaxies in the sample and second because most of the galaxies with $z \sim 1$ were classified¹³ as extended objects on the r CCD identification exposures (equivalent to $\lambda_{\text{rest}} = 3,200 \text{ Å}$) suggesting that they too are not dominated by their nuclei. Part of the motivation for this line of research is to try to relate changes in the stellar population of the galaxy to the well-known changes in the radio properties of the radio source population at increasing redshift, and the present results suggest that star formation and hence presumably interstellar gas, was more plentiful in the past.

Finally, if the trends observed in 3C352 and the other radio galaxies are indicative that evolution similar to that predicted by the Bruzual models is in fact taking place, then there are two important consequences for future work. First, as we go to larger redshifts, the evolutionary effects become greater as the epoch of large scale star formation is approached and as the rest frame wavelengths of standard filters are pushed to shorter and shorter wavelengths. Hence, although radio galaxies with redshifts $z \sim 2$ ought to be impossible to detect optically from the ground, evolutionary changes may be sufficient for these to be detectable relatively easily. Indeed in several of Bruzual's models which seem to be consistent with many of the results to date, the observed B flux density does not decrease at all between redshifts of 0.8 and 2, the cosmological dimming being completely compensated for by evolutionary changes in the stellar population. Second, if the evolution is even stronger in the far UV observed wavebands as will occur in these models, then the far UV deep images of the sky taken by the Space Telescope may prove to be very much more spectacular than we would predict.

We thank members of the ROE ISP team in Edinburgh, the UKIRT group in Hawaii and the Image and Data Processing Unit at ROE for help, Drs M. G. Smith and T. G. Hawarden and P. Hewett for constructive comments, and Photolabs, Edinburgh, for producing the photographic print. S.J.L. acknowledges a Research Studentship from the SERC.

Received 6 October; accepted 8 December 1982.

1. Kristian, J., Sandage, A. & Westphal, J. *Astrophys. J.* **221**, 383–394 (1978).
2. Gunn, J. E. *Proc. Study Week on Cosmology and Fundamental Physics*, 233–262 (Pontificia Academia Scientiarum, 1982).

3. Van der Laan, H. & Windhorst, R. A. *Proc. Study Week on Cosmology and Fundamental Physics*, 263–267 (Pontificia Academia Scientiarum, 1982).
4. Lilly, S. J. & Longair, M. S. *Mon. Not. R. astr. Soc.* **199**, 1035–1068 (1982).
5. McLean, I. S., Cormack, W. A., Herd, J. T. & Aspin, C. *Proc. Soc. photo-opt. Instrum. Engng* **290**, 155–200 (1981).
6. Hine, R. G. & Longair, M. S. *Mon. Not. R. astr. Soc.* **188**, 111–130 (1979).
7. Bruzual, A. thesis, Univ. California, Berkeley (1981).
8. Kristian, J., Sandage, A. & Katem, B. *Astrophys. J.* **191**, 43–50 (1974).
9. Smith, H. E., Junkkarinen, V. T., Spinrad, H., Gruelf, G. & Vigotti, M. *Astrophys. J.* **231**, 307–311 (1979).
10. Spinrad, H. Preprint (Univ. California, Berkeley, 1982).
11. Yee, H. C. & Oke, J. B. *Astrophys. J.* **226**, 753–769 (1978).
12. Ellis, R. S. & Allen, D. Preprint 167 (AAO, 1982).
13. Gunn, J. E., Hoessel, J. G., Westphal, J. A., Perryman, M. A. C. & Longair, M. S. *Mon. Not. R. astr. Soc.* **194**, 111–123 (1981).

Can γ -ray bursts originate from low-mass binaries?

J. Ventura*†, S. Bonazzola‡, J. M. Hameury* & J. Heyvaerts*

* Institut d'Astrophysique, 98 bis bd Arago, 75014 Paris, France
† Lehrstuhl für Theoretische Astrophysik, Universität Tübingen, Auf der Morgenstelle 12, D-7400 Tübingen 1, FRG
‡ Section d'Astrophysique, Observatoire de Meudon, 92190 Meudon, France

γ -Ray bursts have been attributed to binary systems^{1–3} with the burst resulting from an accretion instability^{1,2}, or a thermonuclear explosion at the surface of an accreting magnetic neutron star^{4–6}. Recent deep sky surveys in the X-ray^{7–9} and optical wavelengths (S. Illovaiki and C. Chevalier, personal communication), impose stringent new limitations on the theory. Assuming an average distance of $\sim 300 \text{ pc}$, these observations limit the optical absolute magnitude of bursters in quiescence to $M_v > 13$ and their X-ray luminosity to $< 10^{31} \text{ erg s}^{-1}$ for polar cap accretion (K. Hurley, S. Illovaiki and G. Pizzichini, personal communication). If these are local binary systems in our Galaxy, as suggested by their isotropic distribution in the sky^{10,11}, the binary companion would clearly have to be a very low mass object. Although very peculiar, the presence of such an 'invisible' companion is possibly hinted at by the recently proposed identification of the 19 November 1978 γ -ray burst to a 1928 optical transient event lasting $< 10 \text{ min}$ discovered by Schaefer¹². Motivated by these data, we examine here the possibility of obtaining such low luminosity systems as the evolutionary end products of galactic low-mass binary systems with a neutron star primary.

The evolution of binaries with orbital period, $P < 9 \text{ h}(m/M_\odot)^{3/8}$ (where m is the mass of the dwarf secondary) is determined by gravitational radiation and mass loss¹³, in a manner similar to that proposed for the evolution of cataclysmic variables^{14–18}. In the absence of mass exchange, the system is drawn together until the secondary fills its Roche lobe. Mass exchange begins at this point introducing a tendency to separate the system, as seen from the equation ($m \ll 1M_\odot$)

$$a^{-1} \frac{da}{dt} = -2\dot{m}m^{-1} - \tau_G^{-1} \quad (1)$$

Here $-\dot{m}$ is the mass-exchange rate, a is the orbital separation and τ_G the time scale for orbital decay due to gravitational radiation¹⁹, $\tau_G = 3.9 \times 10^7 \text{ yr} (P/1\text{h})^{8/3} M_\odot m^{-1}$.

The contraction of the system will thus stop at the value $\dot{m} = 3m(4\tau_G)^{-1}$ corresponding to locking the secondary in a semi-detached phase of mass exchange. This solution is fully determined, if we take into consideration the radius-to-mass relation for the secondary. Assuming a $1M_\odot$ primary we thus find that after 10^{10} yr of evolution the secondary has a mass $m = 5 \times 10^{-3} M_\odot (t/10^{10} \text{ yr})^{-3/11}$ and other properties which seem relevant to models for γ -ray bursts: (1) the mass loss rate $\dot{m} = -1.2 \times 10^{-16} M_\odot \text{ yr}^{-1} m_j^{14/3}$ where $m_j = m/10^{-3} M_\odot$; (2) orbital separation $a = 1.3 \times 10^{11} \text{ cm } m_j^{-2/3}$; (3) X-ray luminosity $L_X = 10^{30} \text{ erg s}^{-1} m_j^{14/3}$; (4) optical luminosity of the illuminated

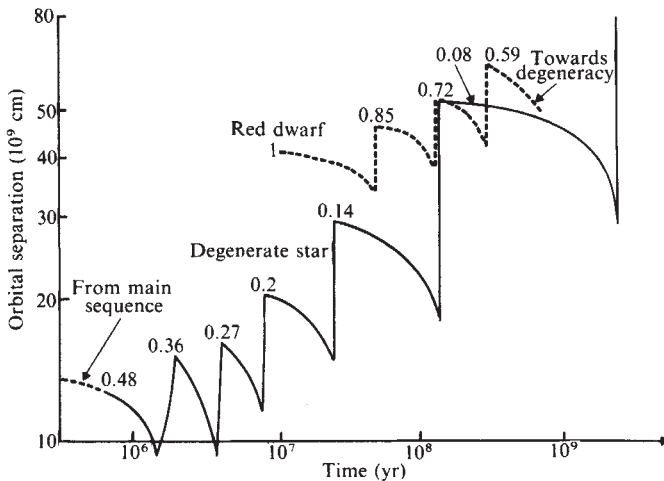


Fig. 1 Evolutionary tracks for a $0.1 M_{\odot}$ main sequence red dwarf (broken line) and for a degenerate star of $0.048 M_{\odot}$ (solid line), which is the assumed continuation of the previous track after cooling and an additional mass loss episode. The evolution in the non-degenerate phase is followed up to the point where the evolution time scale becomes smaller than the Kelvin-Helmholtz time, at which point contraction towards the degenerate state begins. The numbers at the spikes denote the mass of the secondary in units of $0.1 M_{\odot}$. The neutron star primary is assumed to have a constant mass of $1 M_{\odot}$ in our calculation.

secondary $L_2/L_X = 5.5 \times 10^{-4} m_2^{2/3}$ and effective surface temperature $T_2 = 0.5 \times 10^3 \text{ K } m_2^{3/2}$. These expressions are applicable for a helium degenerate secondary in the mass range $1 < m_2 < 100$ satisfying the radius-to-mass relation $20 R \propto m^{-1/3}$.

The stability of Roche Lobe locked scenario has been studied previously^{15,17,18} but no account has been taken of the X-ray illumination of the secondary. On the other hand, the literature on the illumination-induced mass exchange²¹ seems to have an empirical status concentrating on high-mass flow systems, without studying their stability. We shall therefore use a simplified, but physically transparent, argument to study the stability of low-luminosity systems. X-ray heating of the dwarf star results in an increased rate of atmospheric escape through the L1 lagrangian point. This again feeds the accretion on the neutron star, further increasing the X-ray luminosity and may result in a runaway situation. The rate of atmospheric escape has a sensitive nonlinear dependence on the surface temperature and surface gravity of the star²²:

$$\dot{m} = -(2\pi)^{1/2} \rho_0 R^2 (kT/m_A)^{1/2} Y e^{-Y} \quad (2)$$

where $Y = m_A v_{\text{esc}}^2 / (2kT)$, v_{esc} is the velocity of escape necessary to reach the Roche lobe, m_A is the ion mass and ρ_0 the density at the base of the atmosphere taken here to be $\rho_0 = 1 \text{ g cm}^{-3}$, that is, the value at which degeneracy starts to play a part. The temperature T is related to the X-ray illumination: $\sigma T^4 = 0.1 \eta \dot{M} c^2 / (4a)^2$. $\dot{M}$ is the accretion rate on the neutron star, η is an obscuration factor. In our simplified treatment we equate $\dot{M}$ to the mass loss rate of the secondary when this value is less than the Eddington limit and have $\dot{M}$ saturate at the value $\dot{M}_{\text{Edd}}$ otherwise. A linearized analysis of the above equations gives instabilities if the X-ray luminosity 'seen' by the companion exceeds the star's intrinsic luminosity. The development of the instability in time is determined numerically by integrating the nonlinear system of equations.

We find that mass exchange is enhanced even before Roche lobe overflow occurs due to the reduced escape velocity of the atmosphere in the Roche geometry. During the contraction phase X-ray illumination is still operative at a minimum value due to interstellar accretion, $\dot{M}_{\text{min}} \approx 10^{16} M_{\odot} \text{ yr}^{-1}$. The onset of mass exchange soon feeds a thermal instability, as described above, with the mass loss rate rising to the Eddington limit in a very short time. A high mass exchange phase begins at this

point, driving the system apart. The increase in the orbital separation results in an increased effective gravity at the surface of the secondary. The mass supply of the X-ray source is eventually interrupted and the orbital separation begins again to decrease due to the effect of gravitational radiation. Further details of our stability analysis will be given elsewhere. Figure 1 (broken line) shows the evolution for a red dwarf secondary of initial mass $0.1 M_{\odot}$, assumed to be in convective equilibrium^{16,17}. We can follow the change in orbital separation and in mass with the system drawn closer and rebounding during the (interrupted) mass exchange phase three times, before the secondary has time to cool and become a degenerate star of $0.05 M_{\odot}$. The solid line in Fig. 1 shows the evolution of the secondary after it has reached the degenerate phase. We end up again with systems whose secondary has a very low mass ($< 0.01 M_{\odot}$), for which the gravitational time for drawing the system together is of the order of 10^{10} yr . A more sophisticated treatment of the illumination-induced mass exchange should substitute equation (2) by an equation for the hydrodynamic flow through L1²¹. Furthermore, disk obscuration should give the limiting rate of mass exchange in a detailed treatment.

The final configuration in the evolution we propose is a 'detached' system and we therefore conclude that the binary nature cannot be the prime cause of the bursting activity. Binarity can, however, offer definite advantages for two of the currently proposed γ -ray burst models involving galactic magnetic neutron stars. Thus (1) thermonuclear models depend on a minimum accretion rate^{5,6}. Accretion of interstellar matter on the neutron star can be more efficient in the binary compared with accretion on an old, extinct pulsar. This is because the pulsar's fast rotation inhibits the accretion through the propeller mechanism²³, while its expected high peculiar velocity also results in a reduced accretion (a reduced Bondi radius). In contrast, the pulsar's rotation in a binary will be slower as it is controlled by accretion during the last mass-exchange episode. (2) Solid body accretion requires the presence of an asteroid belt^{2,24}. The dwarf companion can provide in this case a perturbation which destabilizes the orbits facilitating an occasional accretion (P. Joss, personal communication).

We have further shown that binaries can be viable candidates for the γ -ray bursts despite the strong observational constraints. This is so, because one can consistently predict low optical and X-ray luminosity objects in the quiescent state at the end of the evolutionary sequence. If, in fact, some of the observed γ -ray bursts have their origin in such binaries, their progenitors would be strong X-ray sources observable at large distances during the high mass exchange phase of their evolution. The rate of confirmed γ -ray bursts is presently measured at about $100 \text{ bursts yr}^{-1}$. Let g be the fraction of the bursts which occur in binary systems as discussed here. One finds the local density of binary burst sources to be $n_b = 2.4 \times 10^{-8} g \text{ pc}^{-3} d^{-3} (\text{kpc})$ $\tau_R (\text{yr})$, where d is the mean distance of the sources and τ_R the recurrence time of bursts from one source. The galactic disk (radius 10 kpc, thickness 600 pc) should then contain $4,500 g d^{-3} \tau_R$ progenitors. These are active X-ray sources during the high mass exchange phase, which we find to cumulate to $6 \times 10^6 f \text{ yr}$, where f is the fraction of the mass lost by the dwarf which is accreted on the compact companion. Hence we expect $3fg d^{-3} \tau_R$ low mass X-ray sources in the Galaxy. With $d \sim 0.3 \text{ kpc}$ (ref 10) and $\tau_R \sim 10 \text{ yr}$ (ref. 6), this amounts to $800 fg$ sources active now. This could be consistent with the observations if f is small (large mass escape from the system) or g is small (few γ bursters are of the type described here). Finally, such binary systems naturally evolve to a close, but detached, configuration with a low mass companion and naturally account for Schaefer's observation of an optical transient counterpart. Actually we calculated that the secondary will be heated during the burst and re-radiate 10^{-3} of the total burst energy in UV and optical radiation in a time of the order of the burst duration itself.

We thank F. Meyer, M. Pakull, H. Ritter and H. C. Thomas for criticism. J.V. acknowledges discussions with J. Trümper

and the hospitality of the Max-Planck-Institut für Extraterrestrische Physik.

Received 8 July; accepted 25 November 1982.

1. Lamb, F. K., Lamb, D. Q. & Pines, D. *Nature phys. Sci.* **246**, 52 (1973).
2. Harwit, M. & Salpeter, E. E. *Astrophys. J. Lett.* **186**, L37 (1973).
3. Sunyaev, R. *French-Soviet Workshop on γ -Ray Bursts*, Paris (1981).
4. Woosley, S. E. & Taam, R. E. *Nature* **263**, 101 (1976).
5. Woosley, S. E. & Wallace, R. K. *Astrophys. J.* **258**, 716 (1982).
6. Hameury, J. M., Bonazzola, S., Heyvaerts, J. & Ventura, J. *Ast. Astrophys.* **111**, 242 (1982).
7. Vedrenne, G. *Phil. Trans. R. Soc. A* **301**, 645 (1981).
8. Hurley, K. *La Jolla Workshop on γ -ray Transients* (1981).
9. Cline, T. L. *et al.*, *Astrophys. J. Lett.* **246**, L133 (1981).
10. Puget, J. L. *Astrophys. Space Sci.* **75**, 109 (1981).
11. Mazets, E. P., Golenetskii, S. V., Aptekar, R. L., Gurian, Yu. A. & Illinskii, V. N. *Soviet astr. Lett.* **6**, 318 (1980).
12. Schaefer, B. E., *Nature* **294**, 722 (1981).
13. van den Heuvel, E. P. J. *X-ray Astronomy* (eds Giacconi, R. & Setti, G.) 115 (Reidel, Dordrecht, 1980).
14. Paczynski, B. *Acta astr.* **17**, 287 (1967).
15. Faulkner, J. *Astrophys. J. Lett.* **170**, L99 (1971).
16. Paczynski, B. *Acta astr.* **31**, 1 (1981).
17. Rappaport, S., Joss, P. C. & Webbink, R. F. *Astrophys. J.* **254**, 616 (1982).
18. Taam, R. G., Flannery, B. P. & Faulkner, J., *Astrophys. J.* **239**, 1017 (1980).
19. Landau, L. D. & Lifshitz, E. M. *The Classical Theory of Fields* (Addison-Wesley, Reading, 1962).
20. Zapolski, H. S. & Salpeter, E. E. *Astrophys. J.* **158**, 809 (1969).
21. Basko, M. M., Hatchett, S., McCray, R. & Sunyaev, R. A. *Astrophys. J.* **215**, 276 (1977).
22. Spitzer, L. Jr in *The Atmosphere of the Earth and Planets* (ed. Kuiper, G. P.) (University of Chicago Press, 1949).
23. Illarionov A. F. & Sunyaev, R. A. *Astr. Astrophys.* **39**, 185 (1975).
24. Colgate, S., Petschek, H. *Astrophys. J.* **248**, 771 (1981).

Does a 2,200 Å hump observed in an artificial carbonaceous composite account for UV interstellar extinction?

Akira Sakata*, Setsuko Wada†, Yoshiji Okutsu*, Haruo Shintani‡ & Yoshikazu Nakada§

Laboratories of * Applied Science and † Chemistry, and ‡ Department of Electronic Engineering, The University of Electrocommunications, Chofugaoka, Chofu-shi, Tokyo 182, Japan
§ Department of Astronomy, Faculty of Science, Tokyo University, Hongo, Bunkyo-ku, Tokyo 113, Japan

The 2,200 Å hump in the interstellar extinction curve has attracted much attention since its discovery by Stecher¹. Graphitic^{2,3} and carbonaceous grains⁴ and MgO (ref. 5) have been considered as possible candidates. Sakata *et al.*⁴ have reported a hump near 2,200 Å in the absorption spectrum of an extract from the Murchison meteorite, a C2 carbonaceous chondrite. The hump was presumed to be due to hydrocarbons having conjugated double bonds. It is highly probable that the interstellar extinction is caused by carbonaceous materials containing such conjugated double bonds. In the present experiment, carbonaceous materials were synthesized by injecting a plasmic gas mixture of hydrogen and carbon into a vacuum chamber. Extinction of the material was measured over the range 1,900–7,000 Å. The extinction profile of the synthesized material clearly exhibits the 2,200 Å hump, the average value for the central wavelength being $2,170 \pm 30$ Å.

The plasmic gas was prepared as follows. Pure methane was introduced into a fused quartz discharge tube and exposed to a microwave field of a magnetron. The plasmic gas thus produced was injected through an orifice into a vacuum chamber. Carbonaceous material condensed on a fused quartz substrate placed directly in the flow. We shall henceforth refer to this substance as quenched carbonaceous composite (QCC). Details of the apparatus have been reported elsewhere⁶.

The initial pressure of methane in the discharge tube was adjusted to 4 torr. Microwave input power was ~ 300 W. H_{α} , H_{β} , C_2 (Swan system) and CH (4,300 Å system) were detected in the emission lines from the plasma. The calculated temperature of electronic excitation of atomic hydrogen was $\sim 6,000$ K. The vibrational temperature of CH was 1,100–1,700 K. QCC was collected at different distances (50, 80 and 140 mm) from the orifice. A dark-coloured solid material began to deposit on the substrate ~ 1.5 min after the initiation of discharge, and the diameter of the circular deposition was 5 mm

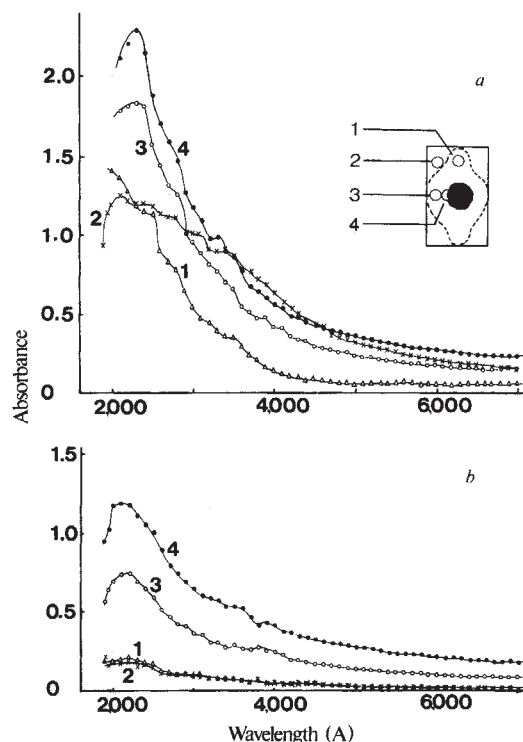


Fig. 1 Absorption spectra of QCC: a, untreated; and b, extracted with MeOH. CH_4 gas at a pressure of 4 torr was discharged with magnetron power of 300 W for 5 min and QCC was obtained on a quartz substrate (30×12 mm) placed 50 mm from an orifice. The numerical values in the inset indicate the position of the measured point on the substrate.

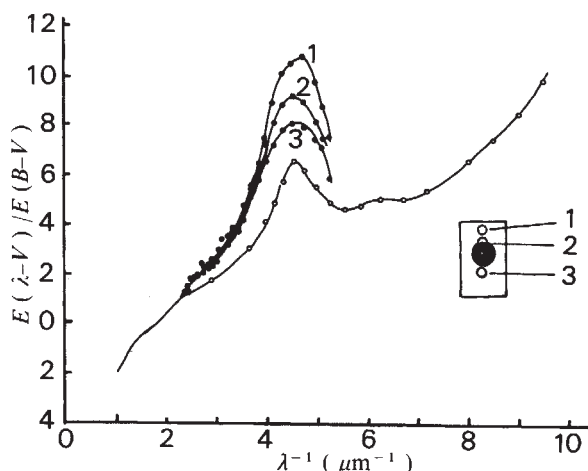


Fig. 2 Normalized extinction curves of QCC (1-3) and the average interstellar extinction curve (open circles). QCC deposition was obtained in the conditions described in Fig. 1 legend.

after 5 min of discharge. The dark area was surrounded by light-brown material. The absorption spectrum of QCC was measured with a circular aperture of diameter 2 mm by using a JASCO spectrometer.

Figure 1a shows typical absorption spectra for QCC. Periodical absorption humps were observed over the range 2,000–3,000 Å (curve 2). These humps indicate the presence of unsaturated organic molecules such as polyynes. These molecules of low molecular weight are expected to be soluble in methanol. Figure 1b shows absorption spectra of QCC after overnight extraction with methanol. The wavelength for the maximum is $2,170 \pm 30$ Å (average of seven points from three samples).

Savage and Mathis⁷ have presented an average normalized interstellar extinction curve from 1 to $10 \mu m^{-1}$ in λ^{-1} . Extinction curves for QCC after methanol extraction were recalculated to give λ^{-1} units on the abscissa, with $B = 4,400$ Å and

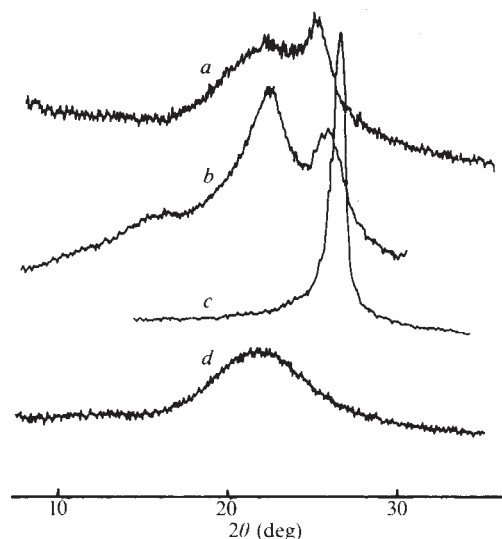


Fig. 3 X-ray diffraction profiles of: *a*, QCC in our experiment; *b*, graphitic material which was produced directly in active discharge of CH_4 ; *c*, naturally occurring crystalline graphite; and *d*, fused quartz (substrate). The distance between the carbon hexagonal layer planes was calculated to be 3.62, 3.47 and 3.39 Å for *a*, *b* and *c*, respectively.

$V = 5,500 \text{ Å}$ for normalization (see Fig. 2). The magnitudes of the maximum extinction vary for different areas of the substrate, in the range 7.7–11. The maximum wavelength shifted slightly towards the shorter wavelength region when the magnitude of the extinction was increased. The general picture of the QCC extinction curves resembles that of the interstellar extinction curve.

The interstellar 2,200 Å hump has a full width at half-strength of 360 Å (ref. 8) to 480 Å (ref. 9). Full width at half-strength for the QCC hump seems a little broader than these values. As our measurements extend only to $5.3 \mu\text{m}^{-1}$, however, there is some uncertainty in setting the baseline for the evaluation of peak height.

X-ray diffraction analysis of QCC revealed a peak at $2\theta = 24.6^\circ$, which indicates the presence of fine graphitic particles (Fig. 3). The distance between the hexagonal layers was calculated to be 3.62 Å. The corresponding value for carbon black (non-graphitized carbon) is 3.4–3.5 Å. The expanded interlayer distance implies that QCC has a turbostratic structure containing some hydrogen atoms.

We believe that in QCC there exist at least two components such as fine graphitic grains and hydrocarbons having conjugated double bonds. Although the scale of our experiment is small compared with interstellar space, we believe we were able to establish experimentally some of the necessary conditions. The distribution of excited states in our plasma was well within the range reported for the photosphere of carbon stars¹⁰. Therefore, the above observation suggests that interstellar grains contain carbonaceous materials which have a similar structure to our QCC.

We thank Mr Kobari for technical assistance and Mr H. Sekiguchi for X-ray diffraction measurements. Quartz substrates (Viosil SMS) were supplied by Shin-Etsu Chemical Co. This study was partly supported by grant 5621007 from the Ministry of Education, Science and Culture of Japan.

Received 12 July; accepted 26 November 1982.

1. Stecher, T. P. *Astrophys. J.* **142**, 1683–1684 (1965).
2. Stecher, T. P. & Donn, B. *Astrophys. J.* **142**, 1681–1683 (1965).
3. Wickramasinghe, N. C. & Guillaume, C. *Nature* **207**, 366–368 (1965).
4. Sakata, A. *et al.* *Nature* **266**, 241 (1977).
5. MacLean, S. & Duley, W. W. *Astrophys. J. Lett.* **256**, L61–L64 (1982).
6. Sakata, A. *IAU Symp.* No 87 325–329 (1980).
7. Savage, B. D. & Mathis, J. S. *A. Rev. Astrophys.* **17**, 73–111 (1979).
8. Nandy, K., Thompson, G. I., Jamar, C., Monfils, A. & Wilson, R. *Astr. Astrophys.* **44**, 195–203 (1975).
9. Savage, B. D. *Astrophys. J.* **199**, 92–109 (1975).
10. Mendoza, V. E. E. & Johnson, H. L. *Astrophys. J.* **141**, 161–169 (1965).

On the reported optical activity of amino acids in the Murchison meteorite

Jeffrey L. Bada*, John R. Cronin†, Ming-Shan Ho*, Keith A. Kvenvolden‡, James G. Lawless§, Stanley L. Miller||, J. Oro¶ & Spencer Steinberg*

* Amino Acid Dating Laboratory, Scripps Institution of Oceanography, La Jolla, California 92093, USA

† Department of Chemistry and Center for Meteorite Studies, Arizona State University, Tempe, Arizona 85287, USA

‡ US Geological Survey, M/S 99, 345 Middlefield Road, Menlo Park, California 92025, USA

§ Ames Research Center, NASA, Moffett Field, California 94035, USA

|| Department of Chemistry, University of California, San Diego, La Jolla, California 92093, USA

¶ Department of Biophysical Sciences, University of Houston, Houston, Texas 77004, USA

In analyses of extracts from the Murchison meteorite (a carbonaceous chondrite), Engel and Nagy¹ reported an excess of L-enantiomers for several protein amino acids but found that the non-protein amino acids were racemic. They suggested that the excess of L-isomers might have resulted from an asymmetric synthesis or decomposition. Their results disagree with those obtained previously^{2–4} and they claim this is due to improved methodology. In fact, their extraction method and analytical procedure (gas chromatography–mass spectrometry, GC–MS) was similar to those used in the original report² of amino acids in the Murchison meteorite except that they used specific ion monitoring in the GC–MS measurements. We found the results of Engel and Nagy odd in that likely contaminants (the protein amino acids ala, leu, glu, asp and pro) were nonracemic while unlikely contaminants (isovaline and α -amino-*n*-butyric acid) were racemic. For example, Engel and Nagy report that the leucine is ~90% L-enantiomer in the water-extracted sample whereas isovaline (α -methyl- α -aminobutyric acid) is racemic. It would be most unusual for an abiotic stereoselective decomposition or synthesis of amino acids to occur with protein amino acids but not with non-protein amino acids. We now show here that the explanation of terrestrial contamination is consistent with their results and is much more probable.

Meteorites are highly susceptible to terrestrial contamination^{5–7}. In fact, there is a general similarity between the enantiomeric ratios of several amino acids in carbonaceous chondrites known to be contaminated with microorganisms^{6–8} and Engel and Nagy's Murchison results. Contamination of the outer part of a meteorite by plant products and soil bacteria at the time of its fall and afterwards by airborne spores is inevitable although some meteorites such as those found in the Antarctic may be less affected.

Organic compounds of terrestrial origin have been found to be localized near the surface of meteorite stones^{9,10}. In the Allende meteorite (a C3 chondrite of the same terrestrial age as the C2 Murchison chondrite) contaminating amino acids were found¹¹ to extend inward from the surface to a depth of between 5 and 8 mm. One would expect contamination at depths at least this great in Murchison stones in view of their surface fissures, grain size, and porosity¹². Simple calculations using Engel and Nagy's sample weights and a meteorite density of 2.86 g cm^{-3} show that these workers initially removed only 0.2 mm from the surface of their stone and that the 39.0 g of 'interior fragments' on which their analyses were done must have contained material that originated as close as 1.4 mm to the original surface, that is, well within the expected zone of terrestrial contamination. Assuming that contaminating amino acids intrude to a depth of 8 mm, then their 50.9-g stone contained a clean core of only 6.6 g.

Table 1 Amino acid enantiomeric ratios in several bacteria and laboratory dust

Sample	D/L ratio				
	ala	leu	glu	asp	pro
<i>Escherichia coli</i> *	0.10	0.05	0.13	0.05	0.01
<i>Photobacterium phosphoreum</i> *	0.10	0.02	0.10	0.05	0.01
<i>Micrococcus euryhalis</i> †	0.39	0.04	0.50	0.05	0.01
Laboratory dust	0.04	0.04	0.05	0.05	0.01

The samples were hydrolysed in 6 M HCl at 100 °C for 24 h. The D/L aspartic acid ratios were determined by HPLC using the diastereomeric dipeptide technique²³. The others were determined by GC using the trifluoroacetyl-L-prolyl chloride method²⁴. The errors in the D/L ratios are ± 0.02 .

* Gram negative. † Gram positive.

Table 2 Calculated percentage of contamination in the Murchison meteorite extracts analysed by Engel and Nagy

Sample	Assumed contamination source	% Of isolated amino acids due to contamination				
		ala	leu	glu	asp	pro
Water extract	50:50 mixture of Gram positive–Gram negative bacteria*	43	86	97	79	65
	50:50 mixture of Gram positive–Gram negative bacteria and laboratory dust†	38	86	85	79	65
HCl extract	50:50 mixture of Gram positive–Gram negative bacteria and laboratory dust†	81	100	100	92	90

The D/L ratios reported by Engel and Nagy were used for $(D/L)_{obs}$ in equation (1).

* Based on the data in Table 1, the enantiomeric ratios for this source of contamination would be: D/L ala = 0.25, D/L leu = 0.04, D/L glu = 0.30, D/L asp = 0.05 and D/L pro = 0.01.

† Based on the data in Table 1, the enantiomeric ratios for this source of contamination would be: D/L ala = 0.15, D/L leu = 0.04, D/L glu = 0.18, D/L asp = 0.05 and D/L pro = 0.01.

Engel and Nagy claim that their excess L-amino acids are not due to contamination because of the low abundance or absence of the amino acids serine, threonine, methionine, tyrosine, and phenylalanine in their extracts. However, these amino acids are not necessarily reliable indicators of the presence of terrestrial contamination for several reasons. Although serine and, to a lesser extent, threonine are important amino acids in fingerprints¹³, they are of only average abundance in proteins and bacteria¹⁴. Moreover, these amino acids are highly susceptible to decomposition during the derivatization procedures used in the GC–MS method. Thus, the analyses of a contaminated meteorite sample might not show substantial quantities of serine and threonine. In any case, one of us (J.R.C.) was able to detect serine in a 0.7 g portion of Engel and Nagy's sample using ion exchange chromatography.

The absence of methionine, tyrosine and phenylalanine is a different problem and can be attributed to various causes. In the case of the hot water extract, the amino acids released by contaminating microbial cells would not be derived from cellular proteins which are stable in these conditions, but rather from the free amino acid pool. Boiling water is commonly used to extract free amino acids in studies of microbial metabolism¹⁵. In bacteria and fungi, the free amino acid content of both spores and vegetative cells may be quite different and generally more restricted than the amino acid composition of their cellular proteins^{15–17}. For example, methionine, tyrosine and phenylalanine have been shown to be absent from the amino acid

pool of bacterial spores¹⁶ and fungi¹⁷. Because the types of contaminating organisms are unknown, it is not possible to predict exactly which amino acids would be added to a meteorite hot water extract by contamination. However, it is possible for microbial cells to be present in a meteorite sample and for methionine, tyrosine, and phenylalanine to be absent from a hot-water extract of that sample.

The amount of contamination in Engel and Nagy's extracts can be estimated from a mass balance of the amino acid enantiomers. For each amino acid

$$(D/L)_{obs} = x(D/L)_{con} + (1-x)(D/L)_{ind} \quad (1)$$

where $(D/L)_{obs}$ is the observed ratio, $(D/L)_{ind}$ is the indigenous ratio taken as 1.0, $(D/L)_{con}$ is the ratio for the contamination and x is the fraction of contamination. The value of x , which will be different for each amino acid, depends on the amount of a given amino acid in the contamination and the indigenous abundance of this amino acid in the meteorite. Thus

$$x = \frac{W_{con}}{W_{con} + W_{ind}} \quad (2)$$

where W_{con} is the weight fraction of a particular amino acid in the contamination (g amino acid contamination per g meteorite) and W_{ind} is the weight fraction of the amino acid which is indigenous to the meteorite (g amino acid per g meteorite).

The $(D/L)_{con}$ values in equation (1) would not be expected to be 0 for all amino acids as D amino acids occur in cell walls of bacteria and some racemization also occurs during extraction and hydrolysis¹⁸. To obtain values for $(D/L)_{con}$ we determined the enantiomeric ratios for several bacterial species, and a sample of laboratory dust; the results are given in Table 1. Using these values to evaluate the enantiomeric ratios associated with various types of contamination and equation (1), it is possible to calculate values of x for the Murchison extracts analysed by Engel and Nagy. These calculations (Table 2) indicate that the amino acids in the HCl extract could have been almost totally derived from contamination while in the water extract the amount of contamination ranges from ~40 to 97% depending on the particular amino acid. The calculated percentage contamination is not sensitive to which source is used as is indicated by the two calculations given for the water extract.

Engel and Nagy explicitly assume that contaminating amino acids will be more easily extracted from the meteorite powder than will indigenous amino acids. They justify this by citing an experiment in which "rocks were soaked in aqueous amino acid solutions" and then extracted by their hot-water procedure. In fact the incorporation of biological amino acids into meteorite specimens as free amino acids may be the exception rather than the rule. Contamination by atmospheric microbes is much more likely. Oró and Tornabene⁷ demonstrated that although some viable bacterial cells can be removed from meteorite samples by soaking in water, most remain attached to the specimen. Our experimental results (Table 2) indicate that the free amino acids in the bacteria contaminating the meteorite were mainly extracted along with indigenous amino acids by the hot water treatment, and that the contaminant protein-bound amino acids were released later on acid hydrolysis of the residue. This is supported by the observation that the protein amino acids are much more abundant than the nonprotein amino acids in the HCl extract. Also, in the water extract alanine has the lowest percentage contamination, which is not unexpected since quantitative amino acid analysis of the indigenous amino acids¹⁹ suggests that W_{ind} for alanine should be greater than those for the other amino acids listed in Table 2. Likewise the highest percentage contamination is found for the amino acid (leucine) present in the lowest concentration in the meteorite.

If the HCl extract of Engel and Nagy contains mainly amino acids from contaminating proteins, why were methionine, tyrosine, and phenylalanine not detected? The failure to detect these amino acids is probably due to both the inadequate sensitivity of their GC–MS method and to the poor recovery of these amino acids. Because Engel and Nagy could not detect

serine and threonine by GC-MS, it is not surprising that they failed to detect methionine, tyrosine, and phenylalanine. Furthermore methionine, tyrosine, and phenylalanine should be among the amino acids of lowest concentration in the HCl extracts because of their low frequency of occurrence in proteins²⁰. As for the recovery problem Engel and Nagy report a control experiment in which substantial but acceptable losses (methionine, 35%; tyrosine, 13%, phenylalanine, 52%) occurred when a solution of these amino acids in water was refluxed with a Murchison meteorite powder, desalted, derivatized, and analysed. This control was carried out at an amino acid to meteorite ratio 100–1,000 times greater than that contained in the meteorite. It is our experience that percentage recoveries decrease at low amino acid-to-meteorite ratios. To demonstrate this, we added 200 nmol of an amino acid standard to 0.5 g of a previously extracted Murchison meteorite sample. This spiked sample was extracted with 2 ml of water at 100 °C for 8 h. The water extract was dried, hydrolysed in 6 M HCl for 24 h at 100 °C, desalted and analysed on the amino acid analyser. Methionine was completely lost and tyrosine and phenylalanine showed extensive decomposition (the loss relative to leucine was ~75% for tyrosine and ~50% for phenylalanine). Losses of methionine and tyrosine can be particularly troublesome due to oxidation of the former to its sulphone and sulphoxide and to chlorination²¹ of the latter when hydrolysis and extraction is carried out in HCl in the presence of air. Even greater losses of these labile amino acids would be expected in the hot HCl extraction because in this case a more complex desalting procedure is required to remove the dissolved iron from the amino acids²².

The results presented by Engel and Nagy can, therefore, be most easily understood if one assumes that their sample had been infected by terrestrial microorganisms and that their processing procedures and analyses simply resulted in the isolation and measurement of this contamination along with the indigenous amino acids.

This work was supported in part by the Alfred P. Sloan Foundation (J.L.B.) and several NASA grants (J.R.C., S.L.M., J.O.). We thank A. Yayanos for providing the bacterial cultures.

Received 23 August; accepted 21 October 1982.

- Engel, M. H. & Nagy, B. *Nature* **296**, 837–840 (1982).
- Kvenvolden, K. A. *et al.* *Nature* **228**, 923–926 (1970).
- Kvenvolden, K. A., Lawless, J. G. & Ponnampertuma, C. *Proc. natn. Acad. Sci. U.S.A.* **68**, 486–490 (1971).
- Lawless, J. G. *Geochim. cosmochim. Acta* **37**, 2207–2212 (1973).
- Hayes, J. M. *Geochim. cosmochim. Acta* **31**, 1395–1440 (1967).
- Oró, J. *et al.* *Nature* **230**, 107–108 (1971).
- Oró, J. & Tornabene, T. *Science* **150**, 1046–1048 (1965).
- Narkaparksin, S. thesis, Univ. Houston (1969).
- Yuen, G. U. & Kvenvolden, K. A. *Nature* **246**, 301–303 (1973).
- Kotra, R. K., Shimoyama, A. & Ponnampertuma, C. in *Origin of Life* (ed. Wolman, Y.) 51–57 (Reidel, Dordrecht, 1981).
- Harada, K. & Hare, P. E. in *Biogeochemistry of Amino Acids* (eds Hare, P. E., Hoering, T. C. & King, K. Jr.) 169–181 (Wiley, New York, 1980).
- Fuchs, L. H., Olsen, E. & Jensen, K. J. *Smithsonian Contributions to the Earth Sciences* No. 10 (1973).
- Oró, J. & Skewes, H. B. *Nature* **207**, 1042–1045 (1965).
- Reeck, G. R. & Fisher, L. *Int. J. Peptide Protein Res.* **5**, 109–117 (1973).
- Holden, J. T. in *Amino Acid Pools* (ed. Holden, J. T.) 73–108 (Elsevier, Amsterdam, 1962).
- Nelson, D. L. & Kornberg, A. *J. Biol. Chem.* **245**, 1128–1136 (1970).
- Bent, K. J. & Morton, A. G. *Biochem. J.* **92**, 260–269 (1964).
- Liardon, R. & Jost, R. *Int. J. Peptide Protein Res.* **18**, 500–505 (1981).
- Cronin, J. R., Gandy, W. E. & Pizzarello, S. in *Biogeochemistry of Amino Acids* (eds Hare, P. E., Hoering, T. C. & King, K. Jr.) 153–168 (Wiley, New York, 1980).
- Doolittle, R. F. *Science* **214**, 149–159 (1981).
- Sanger, F. & Thompson, E. O. P. *Biochim. biophys. Acta* **71**, 468–471 (1963).
- Pollock, G. E., Cheng, C.-N. & Cronin, S. E. *Analyt. Chem.* **49**, 2–7 (1977).
- Bada, J. L. & Protsch, R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1331–1334 (1973).
- Hoopes, E. A., Peltzer, E. T. & Bada, J. L. *J. chromatogr. Sci.* **16**, 556–560 (1978).

Reply by Michael H. Engel* and Bartholomew Nagy†

* School of Geology and Geophysics, The University of Oklahoma, Norman, Oklahoma 73019, USA

† Laboratory of Organic Geochemistry, Department of Geosciences, The University of Arizona, Tucson, Arizona 85721, USA

We previously stressed that “contamination of the Murchison meteorite (is) a serious possibility” and that “further examin-

Table 1 Amino acid abundances for the water extracts of four Murchison meteorite stones

Amino acid	Abundance measured (nm g ⁻¹)				
	Field ¹ museum (Me 2640)	Field ¹⁵ museum (Me 2640)	ASU ^{14,15}	Ames ³	Smithsonian ¹⁵
Ala	15.3	19.4	43.3	33.7	90.7
Leu	1.9	<1.8	<3.9	NR	<1.3
Glu	18.2	15.4	23.5	20.4	109.6
Asp	8.5	4.9	5.1	NR	20.0
Pro	13.5	NR	NR	8.7	NR
Ser	NR	0.9	4.2	NR	9.0

NR, not reported.

ation of amino acids... could be beneficial”¹. Our intent was to encourage independent experimentation rather than speculative assumptions. Bada *et al.*² have postulated speculative and critical comments about our findings and attempted to substantiate these with an erroneous mathematical model that is contingent on assumptions.

With respect to analytical procedures, instrumentation and sensitivity, the ~racemic amino acid D/L values previously reported for the Murchison meteorite are suspect^{3,4}. Inadequate separation of gas chromatographic peaks, baseline interference and harsh extraction and derivatization procedures such as esterification with highly acidic (8M), impure (96%) (+)-2-butanol for excessive time (3 h) at high temperature (110 °C) could have artificially enhanced the reported D/L values^{3,4}. Bada *et al.*² claim that the combined gas chromatography-mass spectrometry used in previous studies^{3,4} was comparable to ours. In our system, however, the enhancement of quasi-parent ions with chemical ionization-mass spectrometry coupled with selected ion monitoring allowed us to determine quantitatively amino acid D/L values, in addition to making qualitative identifications. While inter-laboratory comparison of D/L ratios of amino acids from a terrestrial standard gave divergent values⁵, our findings have been consistently adjacent to the arithmetic mean. We recognize that it is difficult to detect trace amounts of Ser and Tyr in Murchison extracts on Carbowax 20M columns because of inherent baseline interference. This is why J. R. Cronin initially offered to determine Ser and Tyr on his HPLC system; he detected no Tyr (Phe or Met) and only 0.9 nm g⁻¹ Ser, apparently the lowest amount of Ser ever reported for a Murchison stone. Our sensitivity for the non-hydroxy amino acids corresponds to ≤0.1 nm g⁻¹ concentrations. Consequently, we could have detected Phe and Met in our extracts (see ref. 6), if they were introduced by extant microorganism contaminations at the levels required to alter other, for example Glu, D/L values.

According to the model proposed², most contamination is present in the HCl extract of the meteorite, resulting in lower D/L values. As Glu and Ala are major components of bacterial proteins^{7,8}, it is inconsistent with the model that they are more abundant in the portion of the H₂O extract attributed to contamination rather than in the respective portion of the HCl extract (6.6 and 3.9 nm g⁻¹, respectively for Ala and 17.6 and 6.5 nm g⁻¹, respectively for Glu). Also, recent simulation experiments have shown that racemization rates of amino acids recovered by acid extraction from organic, polymeric matrices are retarded⁹, indicating that low D/L values observed for acid extracts of natural systems^{1,10} may not always be attributed to recent contamination. Bada *et al.*² state that Ser, Phe, Thr, Tyr and Met are generally present in low abundances in bacteria and that their absence and/or low abundances in our extracts does not preclude a bacterial contribution. Ser, however, is the seventh most abundant protein amino acid in *Escherichia coli* studied by Bada *et al.*² and Phe, Thr, Tyr and Met are principal components of a wide variety of bacteria (see ref. 8). Ser and Thr are commonly detected in >nm g⁻¹ quantities in meteorites that are thought to be contaminated^{11,12}. Moreover, there are

no reported identifications or counts of viable or dead microorganisms for any of the Murchison stones. Based on the abundances of Ala, Glu, Pro, Asp and Leu attributed to contamination by Bada *et al.* in our stone, other Murchison stones would be more contaminated as they generally contain a greater abundance of these amino acids (Table 1) relative to the 'indigenous' levels in our stone. At least some of the uncommon non-protein amino acids in Murchison may be racemic decomposition products of other amino acids, as has already been reported for terrestrial systems (see ref. 13).

Finally, Bada *et al.*² state that their equation (1) is a mass balance equation. Mass balance equations can be applied to absolute abundances. The use of ratios in equation (1), however, is not valid unless both D_{ind} and D_{con} are $\ll L_{ind}$ and L_{con} . Bada *et al.*² assume, however, that $D_{ind} = L_{ind}$, that $(D/L)_{ind} = 1.0$; their mathematics is, therefore, in error. Consequently, X calculated from equation (1) is greater than X calculated from equation (2) when absolute values for $D_{ind,con,obs}$ and $L_{ind,con,obs}$ are sub-

stituted into the equations. Equation (1) of ref. 2 should be expressed not as ratios in one equation but as absolute values of D- and L-enantiomers in two separate equations, that is $D_{obs} = D_{con} + D_{ind}$ and $L_{obs} = L_{con} + L_{ind}$. For example, Bada *et al.*² report that $X = 0.43$ and $X = 0.81$ for Ala in the H_2O and HCl extracts, respectively. The correct values would be $X = 0.36$ (H_2O) and $X = 0.72$ (HCl), assuming 1) the hypotheses (including one of ours) that $(D/L)_{ind} = 1.0$ and 2) that given the overall diversity in distribution of bacteria and the range of their respective compositions, the three bacteria selected to represent $(D/L)_{con}$ by Bada *et al.*² are in any way representative of what the Murchison meteorite stones may have been exposed to.

We propose that it would be more productive to conduct additional experiments on meteorite amino acids and weigh the validity of the resultant interpretations against each other to attain a higher level of comprehension.

We thank Z. Sofer, J. E. Zumberge, D. Munnecke, and H. Ogino for helpful comments and suggestions.

Received 18 November; accepted 10 December 1982.

- Engel, M. H. & Nagy, B. *Nature* **296**, 837-840 (1982).
- Bada, J. L. *et al.* *Nature* **301**, 494-496 (1983).
- Kvenvolden, K. A. *et al.* *Nature* **228**, 923-926 (1970).
- Kvenvolden, K. A., Lawless, J. G. & Ponnamperna, C. *Proc. natn. Acad. Sci. U.S.A.* **68**, 486-490 (1971).
- Kvenvolden, K. A., in *The Biogeochemistry of Amino Acids* (eds Hare, P. E., Hoering, T. C. & King, K. Jr) 223-232 (Wiley, New York, 1980).
- Nagy, B., Engel, M. H., Zumberge, J. E., Ogino, H. & Chang, S. Y. *Nature* **289**, 53-56 (1981).
- Lehninger, A. L. *Biochemistry* (Worth, New York, 1972).

- Block, R. J. *Amino Acid Handbook, Methods and Results of Protein Analysis* (Thomas, Springfield, Illinois, 1956).
- Engel, M. H. & Hare, P. E. *Yb. Carnegie Instr. Wash.* **81**, 425-430 (1982).
- Hoering, T. C. in *The Biogeochemistry of Amino Acids* (eds Hare, P. E., Hoering, T. C. & King, K. Jr) 193-201 (Wiley, New York, 1980).
- Oro, J., Nakaparksin, S., Lichtenstein, H. & Gil-av, E. *Nature* **230**, 107-108 (1971).
- Harada, K. & Hare, P. E. in *The Biogeochemistry of Amino Acids* (eds Hare, P. E., Hoering, T. C. & King, K. Jr) 169-181 (Wiley, New York, 1980).
- Bada, J. L., Steinberg, S., Ho, M.-S. & Ruttenberg, K. *Geol. Soc. Am. Abstr. Prog.* **14**, 437 (1982).
- Cronin, J. R., Pizzarello, S. & Moore, C. B. *Science* **206**, 335-337 (1979).
- Cronin, J. R. & Pizzarello, S. (in preparation).

Metallic glasses as novel catalysts

W. E. Brower Jr, M. S. Matyjaszczyk, T. L. Pettit & G. V. Smith

Southern Illinois University, Carbondale, Illinois 62901, USA

Metallic glasses are nonequilibrium amorphous metal alloys which combine the metallic electronic structure with a disordered atomic structure usually reserved for ceramic and polymeric materials. Amorphous silicon-based structures have already been shown to be effective in semiconductor^{1,2} and photovoltaic applications³, where single crystal structures were previously thought to be required. The properties of metallic glasses have been reviewed recently by Gilman⁴ and Turnbull⁵. Recent reports demonstrate that metallic glasses are indeed both catalytically active⁶ and uniquely selective⁷. Here we disclose the nature of this unique selectivity. We report observations of glassy Pd-Si and Pd-Ge alloys which exhibit different selectivity in catalysis of hydrogen reactions than their crystalline Pd systems. We also observe an increase in the activity of these amorphous catalysts when partial crystallization occurs.

A rapid solidification technique known as splat cooling produces palladium-based metallic glasses. Using isothermal vacuum ageing techniques we have found these glasses to be metastable at and above 300 °C, in good agreement with Chen and Turnbull⁸. The splat-cooled glassy phases in both $Pd_{80}Si_{20}$ and $Pd_{77}Ge_{23}$ which we have produced have been reported previously^{7,9}. The physical forms of glasses produced by the shock-tube type splat cooling device, which we have used¹⁰ are thin flakes (2-10 μm) with a surface area of 0.055 $m^2 g^{-1}$ as measured by the Brunauer, Emmett and Teller method using krypton adsorption. The flakes are nonporous as shown by scanning electron microscopy, exposing a surface area similar to their geometric surface area.

The metastable phases in the Pd-Si and Pd-Ge systems which were studied in this work are listed in Table 1, along with a summary of their catalytic behaviour. The two-phase metastable structures, glass plus incipient crystallization as detected

by X-ray diffraction and electron diffraction, have been reported previously^{9,11}. We observed incipient crystallization in unaged $Pd_{80}Si_{20}$ splats, whereas Masumoto and Maddin¹¹ produced incipient crystallization by isothermal ageing. Duwez⁹ also observed incipient crystallization in unaged $Pd_{77}Ge_{23}$ splats. The Pd-Si glassy phase, the partially crystallized Pd-Si glassy phase, and the partially crystallized Pd-Ge glassy phase, all exhibit catalytic activity in the dissociation of hydrogen/deuterium and in the hydrogenation and deuterium exchange of *cis*-cyclododecene.

Experiments were conducted using an apparatus as previously described⁷ in the liquid phase at 25 °C by shaking a mixture of 0.5 g *cis*-cyclododecene and between 0.04 and 0.08 g of splats under deuterium at a pressure of 1 atm. Analyses and separations were by gas chromatography. No special precautions were taken to eliminate possible mass transport problems and the activities in Table 1 are calculated from plots of deuterium consumed against time. These activities differ from those over both the conventional catalysts and pure crystalline Pd in the following ways: first, the glassy and partially crystallized glassy catalysts produce a higher ratio of isomerization to hydrogenation; and second, they produce a different distribution of exchanged molecules. Ordinarily, over crystalline palladium catalysts, *cis-trans* isomerization of carbon-carbon double bonds is fast. It was particularly fast in these experiments because the less stable of the two isomers was isomerizing to the more stable. That is, a large amount of *trans* isomer was produced along with the saturate. However, there was a difference over the glasses. They produced more *trans* isomer than their crystalline relatives. Also, the glasses produce different deuterium distributions in both the exchanged cyclododecenes and the cyclododecane (Tables 2, 3, Fig. 1). In Table 2, for example, relative to palladium powder (expt 28), Pd/Si glass (expt 30) produces more monodeutero-*trans*-cyclododecene (52.6% compared with 40.6%); concomitantly, in Table 3, the glass produces more dideuterocyclododecene (expt 30, 31.7%; expt 28, 24.1%).

The addition of Si and Ge to form Pd-based glasses alters both the catalytic selectivity and activity from that of pure, crystalline Pd. These differences could arise from an altered surface electronic band structure and/or from an altered surface topography. The lack of 3d electrons in Si, as compared with

Table 1 Phases produced in splat-cooled and conventionally produced palladium and their catalytic behaviour

Alloy	Phases present	Catalyst form	Condition	Expt no.	Activity (specific rate*)	Selectivity % d ₁ [†] % d ₂ [‡]	
Pd-20a/oSi	Glass	Flakes	As splat cooled	30	8.0	52.6	31.7
Pd-20a/oSi	Glass and incipient crystallization	Flakes	As splat cooled	46	15.8	44.6	25.7
Pd-23a/oGe	Glass and incipient crystallization	Flakes	As splat cooled	33	8.2	54.7	35.3
Pure Pd (99.99)	f.c.c.	60 mesh powder	As received	28	12.1	40.6	24.1
Pure Pd	f.c.c. (large crystallites)	5% Pd on charcoal ¹⁸	As received	0	—	33.8	27.3

* Product molecule per min per surface Pd atom after Hussey²⁰. Calculated using plots of deuterium consumed against time.

† Per cent monodeutero *trans*-cyclododecene.

‡ Per cent dideuterocyclododecene.

Table 2 Examples of deuterium distributions in *trans*-cyclododecene isomerized from *cis*-cyclododecene

Catalyst (% <i>trans</i> ¶)	Pd/C* (23.9%)		Pd powder† (14.5%)		Pd-Si‡ (14.2%)		Pd-Ge§ (10.9%)		Pd-Si (24.1%)	
Expt no.	Expt 0	Ran. #	Expt 28	Ran.	Expt 30	Ran.	Expt 33	Ran.	Expt 46	Ran.
d ₀	48.0	43.9	36.0	28.0	24.5	27.0	27.8	27.2	31.7	29.2
d ₁	33.8	37.7	40.6	40.6	52.6	28.9	54.7	41.9	44.6	37.3
d ₂	12.5	14.6	9.9	23.6	12.8	24.0	13.7	24.2	13.3	22.4
d ₃	3.8	3.3	5.6	6.8	4.6	8.2	5.9	6.2	4.8	8.4
d ₄	1.1	0.5	4.2	1.0	2.4	1.7	2.9	0.6	2.2	2.2
d ₅	0.3	0.1	3.7	0.1	1.3	0.2	—**		1.1	0.4
d ₆	0.1	0.0	—**		1.0	0.0			0.7	0.1
d ₇	0.1	0.0			0.7	0.0			0.4	0.0
d ₈	0.1	0.0							0.3	0.0
d ₉									0.2	0.0
d ₁₀									0.2	0.0
d ₁₁									0.1	0.0
d ₁₂									0.1††	0.0
d _{avg.}	0.8		1.1		1.2		1.1		1.2	

* Ref. 16.

† Crystalline; 99.99%, 60 mesh, approximately same surface area as splats.

‡ Pd₈₀Si₂₀ glassy splat.

§ Pd₇₇Ge₂₃ partially glassy splat.

|| Pd₈₀Si₂₀ splat exhibiting incipient crystallinity.

¶ Percentage *trans* isomer in reaction mixture.

* Random distribution calculated from binomial for d_{avg.} distributed among total number of positions shown experimentally to contain deuterium.

** Mass spectrum disappears into background here because of small sample used.

†† Up to d₁₇ found.

Table 3 Examples of deuterium distributions in cyclododecene from hydrogenation with deuterium (deuteriumation) of *cis*-cyclododecene

Catalyst (% saturate¶)	Pd/C (32.6%)		Pd Powder (8.5%)		Pd-Si (8.0%)		Pd-Ge (7.1%)		Pd-Si (13.2%)	
Expt no.	Expt 0	Ran.	Expt 28	Ran.	Expt 30	Ran.	Expt 33	Ran.	Expt 46	Ran.
d ₀	19.1	18.9	18.0	9.2	11.7	10.3	8.5	11.0	17.1	12.6
d ₁	38.1	34.6	32.1	24.1	34.2	26.6	35.6	28.6	36.5	27.6
d ₂	27.3	28.1	24.1	29.1	31.7	30.6	35.3	31.7	25.7	28.6
d ₃	9.0	13.3	8.8	21.5	9.1	20.6	10.2	19.6	8.4	18.7
d ₄	3.1	4.1	5.1	10.9	4.5	8.9	4.7	7.3	4.1	8.6
d ₅	1.8	0.8	3.2	3.9	2.7	2.6	2.8	1.6	2.3	3.0
d ₆	0.6	0.1	2.1	1.1	2.0	0.5	1.8	0.2	1.5	0.8
d ₇	0.4	0.0	1.7	0.2	1.5	0.1	1.3	0.0	1.0	0.2
d ₈	0.3	0.0	1.1	0.0	1.4	0.0	—		0.7	0.0
d ₉	0.2	0.0	1.0	0.0	1.2	0.0			0.6	0.0
d ₁₀			0.9	0.0	—				0.5	
d ₁₁			0.8	0.0					0.4	
d ₁₂			0.7	0.0					0.3	
d ₁₃			0.5	0.0					0.2	
d _{avg.}	1.5		2.1		2.0		1.9		2.0	

Details as described in Table 2 footnotes, except ¶, which represents percentage cyclododecene in reaction mixture.

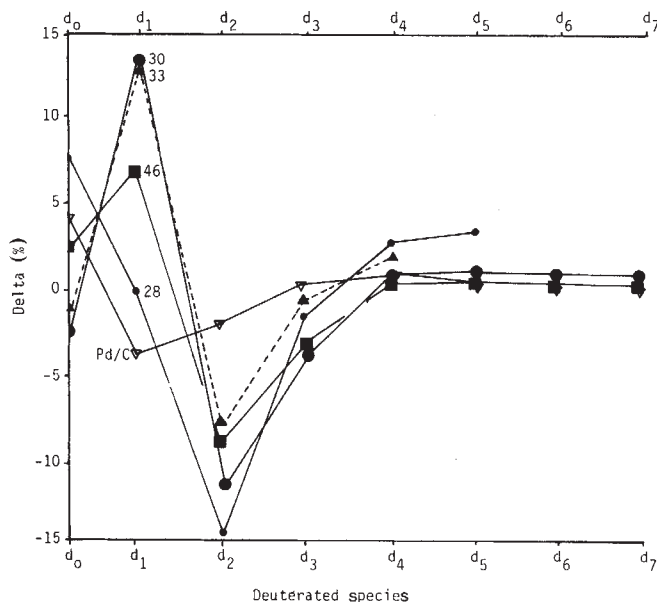


Fig. 1 Deviations of experimental deuterium distributions from random distributions in *trans*-cyclododecene obtained from *cis*-cyclododecene isomerization over glassy and crystalline palladium catalysts. Ordinate (delta values) is difference between experimental and random percentages in Table 2, for example, the value for the undeuterated species, d_0 , from expt 28 is $36.0 - 28.0 = 8.0$ and for the monodeutero species, d_1 , 0.0. Expt 28, Pd powder; expt 46 Pd/Si glassy splat exhibiting incipient crystallinity; expt 30, Pd/Si glassy splat; expt 33, Pd/Ge partially glassy, partially crystalline splat.

the full $3d^{10}$ shell in Ge, should alter the band structure of the parent Pd differently. The hydrogenation results, however, show little difference between the $Pd_{77}Ge_{23}$ and $Pd_{80}Si_{20}$ glassy structures (Fig. 1, expts 33 and 30 respectively). The glassy and partly glassy structures all (Pd-Si and Pd-Ge) show a different selectivity for deuterium exchange from the non-glassy, crystalline structures—pure Pd powder, Pd-Si master alloy powder, and crystallized (produced by isothermal vacuum ageing) alloy splats⁷. This comparison suggests that the electronic or topographic surface structure of the crystalline alloy may be significantly different from the parent glass. However, examination of the surface electronic structure via both Auger electron spectroscopy (AES) and UV photoelectron spectroscopy (UPS) show no difference in the spectra between the glassy splat flakes and these same flakes fully crystallized *in situ*¹². The UPS spectra were compared up to 12 eV above E_t and the AES spectra were analysed in the range 70 to 120 eV. This is in agreement with Gaskell's model of the $Pd_{80}Si_{20}$ glass¹³, in which the short-range order of the glass is similar to that in crystalline $Pd_{80}Si_{20}$. The remaining possible difference between the glassy and crystalline structures is surface topography. Comparative models of a crystalline surface and a glassy surface show the glassy surface to be free of atomically flat terraces and to be highly populated with protuberances approximating kinks and ledges, as compared with the surfaces of large crystallites where the terraces predominate¹⁴. The kinks and ledges on crystalline surfaces are of discrete dimensions¹⁵, whereas the glassy surfaces present protuberances with a continuum of coordination numbers. These surface structural differences may account for our experimental results. For example, addition (Horiuti-Polanyi hydrogenation) is believed to predominate on kinks¹⁶ which are relatively coordinatively unsaturated¹⁷. The isomerization of *cis* to *trans*-cyclododecene can occur in two ways—by the Horiuti-Polanyi mechanism¹⁸ on ledge and kink sites and by a 1,3-sigmatropic hydrogen shift¹⁹ on terrace sites¹⁶. The hydrogen shift isomerization produces d_0 -*trans*-cyclododecene, while the Horiuti-Polanyi isomerization produces d_1 -*trans*-cyclododecene. Therefore, the relative dearth of terraces on the glasses should cause a lower yield of d_0 -*trans*-cyclododecene

and a higher yield of d_1 -*trans*-cyclododecene. Table 2 and Fig. 1 (expt 30 versus expts 46 and 28) reveal this to be the case and confirm the lack of terraces on the glasses.

Glassy metals thus appear to offer a new dimension in the catalysis of organic reactions. They exhibit a selectivity not readily available to crystalline catalysts, while maintaining the turnover frequency²⁰ characteristic of the noble metal catalysts.

This work was supported through contract DE-ACO2-81ER 10897 from the US Department of Energy, Division of Materials Sciences, Office of Energy Research.

Received 1 July; accepted 15 November 1982.

1. Mott, N. F. *J. non-cryst. Solids* **1**, 1–17 (1968); *Adv. Phys.* **16**, 49–144 (1967).
2. Mott, N. F. & Twose, W. D. *Adv. Phys.* **10**, 107–163 (1961).
3. Ovshinsky, S. R. & Madan, A. *Nature* **276**, 482–483 (1978).
4. Gilman, J. J. *Science* **208**, 856–861 (1980).
5. Turnbull, D. *Met. Trans.* **12B**, 217–230 (1981).
6. Masumoto, T. & Kimura, H. M. *J. Catalysis* **68**, 355–361 (1981).
7. Smith, G. V., Brower, W. E., Matyjaszczyk, M. S. & Pettit, T. L. *Proc. 7th int. Congr. Catalysis* (eds Seiyana T. & Tanabe, K.) 355–363 (Elsevier, New York, 1981).
8. Chen, H. S. & Turnbull, D. *Acta metallurgica* **17**, 1021–1031 (1969).
9. Duwez, P. *Trans. A. S. M.* **60**, 605–633 (1967).
10. Klement, W., Willens, R. H. & Duwez, P. *Nature* **187**, 869–870 (1960).
11. Masumoto, T. & Maddin, R. *Acta metallurgica* **19**, 725–741 (1971).
12. Bader, S. D., Richter, L., Brodsky, M. B., Brower, W. E. & Smith, G. V. *Solid St. Commun.* **37**, 729–732 (1981).
13. Gaskell, P. H. *Nature* **276**, 484–485 (1978).
14. Matyjaszczyk, M. S. thesis, Southern Illinois Univ. (1981).
15. Somorjai, G. A. *Adv. Catalysis* **26**, 1–68 (1977).
16. Ledoux, M. J. & Gault, F. G. *J. Catalysis* **60**, 15–20 (1979).
17. Siegel, S., Outlaw, J. Jr & Garti, N. *J. Catalysis* **52**, 102–115 (1978).
18. Smith, G. V. & Menon, M. C. *Ann. N.Y. Acad. Sci.* **158**, 501–509 (1969).
19. Smith, G. V. & Swoap, J. R. *J. org. Chem.* **31**, 3904–3906 (1966).
20. Hussey, A. S. & Nowack, G. P. *J. org. Chem.* **34**, 439–444 (1969).

Supposed Permo-Triassic megashear between Laurasia and Gondwana

A. Hallam

Department of Geological Sciences, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK

A consensus has emerged that during the Palaeozoic several landmasses progressively collided to form the supercontinent Pangaea, which subsequently split up in the Mesozoic and Tertiary to produce the present array of continents. But whereas there is a high measure of agreement about the general shape of the early Jurassic Pangaea, shortly before the initial Atlantic opening, three very different reconstructions of the end-Palaeozoic supercontinent have recently been proposed. These differences, which involve the relative positions of the northern and southern sectors of Pangaea, known respectively as Laurasia and Gondwana, relate to the interpretation of palaeomagnetic data. The three constructions are here tested with reference to geological and palaeobiogeographic data and arguments.

There has been a major problem concerning the interpretation of Permian palaeomagnetic results, which have persistently suggested a lack of coincidence between the polar wander paths of Laurasia and Gondwana; if the geocentric axial dipole model is correct, unacceptable overlap can apparently be avoided only by invoking a considerable right lateral offset. Vine¹ has suggested that there may have been large non-dipole components of the Permian geomagnetic field, and other palaeomagnetists² have argued that the data can be reconciled with what can be called the conventional Pangaea reconstruction (Fig. 1a) which was accordingly adopted in the Palaeozoic map-making exercise of Scotese *et al.*³.

The new data analysis of Morel and Irving⁴ suggests, however, that what they term Pangaea A was only in existence from late Triassic to early Jurassic times. If the concept of an immobile Pangaea back into the Permian is correct, it implies that the palaeomagnetic pole positions for many pre-Jurassic rocks may be subject to unacceptably large errors of up to 15°. The solution put forward to the dilemma is to postulate a significant right

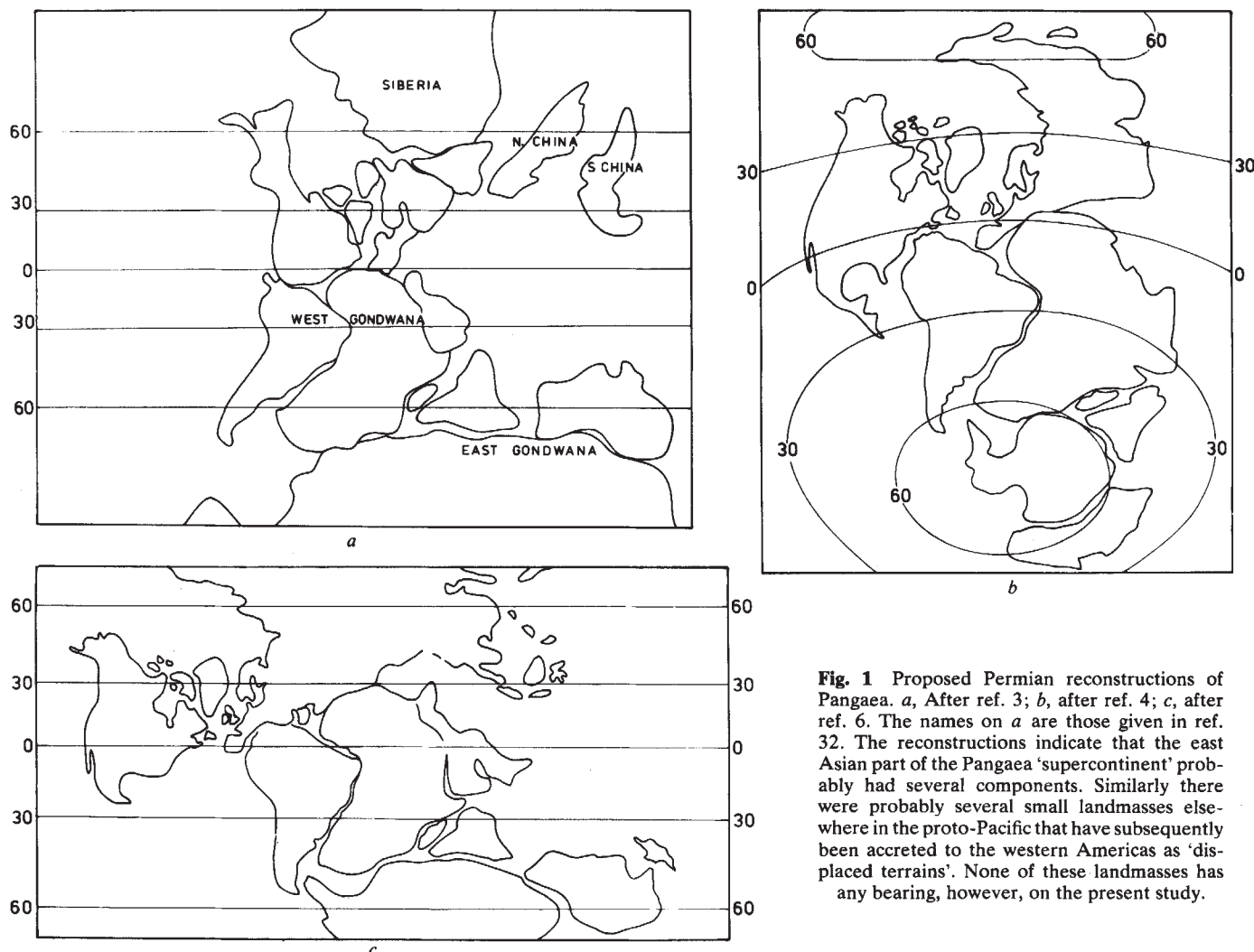


Fig. 1 Proposed Permian reconstructions of Pangaea. *a*, After ref. 3; *b*, after ref. 4; *c*, after ref. 6. The names on *a* are those given in ref. 32. The reconstructions indicate that the east Asian part of the Pangaea 'supercontinent' probably had several components. Similarly there were probably several small landmasses elsewhere in the proto-Pacific that have subsequently been accreted to the western Americas as 'displaced terrains'. None of these landmasses has any bearing, however, on the present study.

lateral offset between Laurasia and Gondwana, with South America aligned against eastern North America and north-west Africa against southern Europe in Permian times; Morel and Irving call this Pangaea B (Fig. 1*b*). A similar late Palaeozoic configuration is proposed by Kanasevich *et al.*⁵.

In view of their new analysis of the palaeomagnetic data Smith *et al.*⁶ have abandoned an earliest Pangaea A reconstruction for the Permian⁷ in favour of one, here termed Pangaea C (Fig. 1*c*), that involves an even greater offset than Pangaea B, with South America being originally situated opposite southern Europe and northern Africa against southern Asia; they consider Pangaea B to be an unsatisfactory compromise.

The most obvious test of the three rival reconstructions is to attempt a match of the geology between adjacent sectors of northern and southern continents in the different positions. The rocks and structures compared must be pre-Triassic and preferably Permian or late Carboniferous, because it is widely believed that earlier in the Palaeozoic oceans intervened. Unfortunately in many of the critical areas along the Tethyan zone the Palaeozoic record has been obscured by the overprint of younger events but sufficient data are, nevertheless, available to make relevant comparisons with some confidence. In addition, the bearing on the problem of some late Palaeozoic faunal and floral distribution patterns can be assessed and structural implications evaluated.

The Pangaea A reconstruction can be tested by comparing the relevant geology of the following regions.

(1) *Northern South America—Central and North America.* A Hercynian orogenic belt can be traced from the Ouachita Mountains southwards into Mexico^{8,9}, with a likely continuation in the Venezuelan and eastern Cordillera of the Colombian

Andes, where a major post-early Permian-pre-Triassic orogenic and metamorphic episode is recorded¹⁰. Taphrogenic tectonics and continental red bed deposition characterize the early Mesozoic of the extensive Atlantic border zone from Nova Scotia to northern South America. The late Carboniferous and Permian of the eastern Colombian and Venezuelan Andes (Perija Range) is marine, as in Guatemala and southeastern Mexico¹¹, and the invertebrate fauna of the Lower Permian carbonates of the Perija Range closely resembles that of the classic Delaware Basin sequence of New Mexico and West Texas¹². There was a major orogenic and metamorphic pre-Devonian, approximately 'Caledonian', episode in the northern Andes¹⁰.

(2) *North-west Africa—Iberia.* In contrast to the regions mentioned above the youngest marine Palaeozoic in these two regions is Viséan. Another difference from the northern Andes is that the Caledonian orogeny was, unlike the Hercynian, of minor importance^{13,14}. With clockwise rotation of Iberia to close the Bay of Biscay, there is only a slight bend in the strike of the Hercynian fold belt from Iberia to Morocco, and there is a broad similarity of late Palaeozoic facies^{15,16}. Pangaea A is, furthermore, the only model to permit the late Palaeozoic symmetry of the Hercynian fold belt of Spain. More general similarities of Palaeozoic rocks, structures and faunas between Iberia and north-west Africa have been noted¹⁷.

(3) *North-west Africa—eastern North America.* The Palaeozoic of the Mauretides, extending from Morocco to Sierra Leone, exhibits many similarities to that of the Appalachian mountain belt, which in many respects is a mirror image of the Mauretides¹⁸. The Lower Palaeozoic Meguma Group of southeastern Canada is sufficiently similar to a complex in the

Table 1 Summary of evidence favouring Pangaea A reconstruction**Geological matching**

- (1) Marine Upper Carboniferous and Permian of northern Andes very similar to central America and southern USA.
- (2) Strong similarities in late Palaeozoic facies and structures between Morocco and Iberia. Post Lower Carboniferous deposits continental, unlike in the Americas.
- (3) Strong Palaeozoic facies similarities between Nova Scotia and Morocco, and between south-east USA and north-west Africa.

Faunas

- (4) Strong resemblances of Permian marine invertebrates between northern Andes and central America-southern USA.
- (5) 'Pacific' and 'Appalachian' elements in late Palaeozoic faunas of western South America.
- (6) Late Palaeozoic terrestrial floras of north-west Africa match European, not central Asian province.

Structural arguments

- (7) Evidence of taphrogeny, not strike-slip motion, in relevant regions during the critical late Permian-early Triassic time interval.
- (8) Implausibility of inferred rates of shear movement between supercontinents, and irregularity of junction between them.
- (9) Structural models involving strike-slip movement, and collision to produce Mauretanides-Appalachians, inconsistent with Pangaea B and C reconstructions.

Moroccan Atlas to suggest to Schenk¹⁹ an African derivation. An African source is also inferred for non metamorphic Lower Palaeozoic subsurface deposits in Florida on the grounds of their trilobite fauna^{20,21}. Suturing during a Hercynian collision event, followed by Mesozoic Atlantic opening along a slightly different line, has been invoked to account for this, and for the opposed directions of overthrusting of the two opposed mountain belts²².

Thus, for all three sets of comparisons, there are many salient geological facts that are consistent with a Pangaea A reconstruction for the Permian. Pangaea B and C, in contrast, produce anomalies, the most notable of which is the absence of post-Visean marine Palaeozoic deposits in north-west Africa and south-west Europe. Pangaea C suffers from the additional major drawback that no continent-continent collision can be invoked to account for the Hercynian compressional tectonics of the Appalachians and Mauretanides.

Disregarding the faunal similarities already noted, only a limited amount of pertinent palaeobiogeographic evidence is available and is hence indecisive, but it appears to support the Pangaea A reconstruction rather than the alternatives. Thus the early Permian fusulinid genus *Dunbarinella* has been recorded only from eastern Asia, western North America and Peru, and defines a distinctive 'Pacific' element²³. This pattern resembles that found in the late Triassic and Jurassic^{24,25} and is much more difficult to account for with a Pangaea B or C than with a Pangaea A reconstruction. The same applies to early Devonian brachiopod faunas, with eastern North America and northwestern South America belonging to the Appalachian Province whereas Europe and north-west Africa belong to the so-called Old World Province²⁶. With regard to late Carboniferous-early Permian terrestrial floras, North Africa forms part of the Euramerican Province, embracing Europe and eastern North America, whereas central Asia (east of the Urals) belongs to the quite different Angara Province²⁷. In the Pangaea C reconstruction, however, North Africa is placed adjacent to south central Asia.

The third line of argument concerns the structural implications of the Pangaea B and C reconstructions. Morel and Irving⁴ demand a minimum 3,500 km continent-continent right lateral shear movement in late Permian and Triassic time, between ~250 and 200 Myr, that is an average rate of 7 cm yr⁻¹. This is an unusually high rate of movement compared with most Cenozoic seafloor spreading rates. It is, nevertheless, greatly exceeded by the rate of movement that must be invoked for the Smith *et al.*⁶ proposal that Pangaea C was converted to Pangaea A between the late Permian (Tatarian) and early

Triassic (Anisian). This implies movement of ~6,000 km in a mere 20 Myr and gives the astonishingly high average rate of 30 cm yr⁻¹. Smith *et al.* concede as the less favoured alternative that movement might have continued until the late Triassic (~200 Myr) which has the effect of reducing the rate to 15 cm yr⁻¹.

Because continent-continent shear is involved, such high rates of movement would be expected to leave a marked structural imprint on the adjacent parts of Laurasia and Gondwana. Oblique plate interactions are known to be characterized by a distinctive suite of features such as extensive strike-slip faults, which bend and ramify into segments active at different times. At the bends, extension or compression give rise to ephemeral basins with a complex sedimentary history, and to rotation of crustal blocks. There may also be sets of *en echelon* periclinal faults oriented at low angles to the principal faults²⁸.

Claims have been made, on the basis of such evidence, that the European Hercynides are indeed an orogen produced by right lateral movement between the northern and southern continents^{17,29,30}. Major strike-slip movement had clearly ceased, however, by mid-Permian times, and this type of tectonic regime was replaced in both Europe and North Africa in late Permian and early Mesozoic times by a taphrogenic regime characterized by an irregular pattern of horsts, grabens and half grabens, with local alkaline volcanicity^{14,30,31}. Extensional tectonics also characterize the early Mesozoic (and late Permian?) of eastern North America, central America and northern South America. This extensive taphrogenic regime anticipated the mid-Jurassic opening of the central sector of the Atlantic Ocean.

It follows that substantial strike-slip movement cannot plausibly be invoked for the time interval required on the basis of palaeomagnetic evidence. A further difficulty is that, whereas well known major strike-slip faults such as the Alpine of New Zealand and the San Andreas of California are essentially straight for distances of hundreds of kilometres, the line of contact between Laurasia and Gondwana is anything but straight over such large distances. Furthermore, Pangaea A is the only model to keep the Atlas Fault of North Africa parallel to the strike of the orogen and the other major Hercynian faults. In the other models it cuts diametrically across the fold belt and other faults, and would have done so at a time of activity on all these faults.

In effect, the oblique collision models of Badham¹⁷, Arthaud and Matte²⁹ and Dewey³⁰ are compatible only with Pangaea A and totally at variance with B and C. The arguments that favour the Pangaea A reconstruction are summarized in Table 1.

In conclusion, there are good geological grounds, supported by palaeobiogeography, for rejecting the Pangaea B and C reconstructions for Permo-Triassic times. The palaeomagnetic data held to support these reconstructions should be re-evaluated in the light of geological constraints.

I thank Dr J. P. N. Badham for helpful comments.

Received 21 October; accepted 8 December 1982.

1. Vine, F. J. *Spec. Pap. Palaeont.* **12**, 61-77 (1973).
2. van der Voo, R. & French, R. B. *Earth Sci. Rev.* **10**, 99-119 (1974).
3. Scotese, C. R., Bambach, R. K., Barton, C., van der Voo, R. & Ziegler, A. M. *J. Geol.* **87**, 217-277 (1979).
4. Morel, P. & Irving, E. *J. geophys. Res.* **86**, 1853-1872 (1981).
5. Kanasevich, E. R., Havskov, I. & Evans, M. E. *Can. J. Earth Sci.* **15**, 919-955 (1978).
6. Smith, A. G., Hurley, A. M. and Briden, J. C. *Phanerozoic Palaeocontinental World Maps* (Cambridge University Press, 1980).
7. Smith, A. G., Briden, J. C. & Drewry, G. E. *Spec. Pap. Palaeont.* **12**, 1-42 (1973).
8. Murray, G. E. *Geology of the Atlantic and Gulf Coastal Province of North America* (Harper & Row, New York, 1961).
9. Dengo, G. in *The Ocean Basins and Margins* Vol. 3 (eds Nairn, A. E. M. & Stehli, F. G.) 325-327 (Plenum, New York, 1975).
10. Shagam, R. in *The Ocean Basins and Margins* Vol. 3 (eds Nairn, A. E. M. & Stehli, F. G.) 325-327 (Plenum, New York, 1975).
11. de Cserna, Z. in *The Encyclopedia of World Regional Geology* Part I (ed. Fairbridge, R. W.) 348-360 (Dowden, Hutchinson & Ross, Stroudsburg, 1975).
12. Trümpy, D. *Bull. geol. Soc. Am.* **54**, 1281-1304 (1943).
13. Rios, J. M. in *The Ocean Basins and Margins* Vol. 4B (eds Nairn, A. E. M., Kanes, W. H. & Stehli, F. G.) 1-65 (Plenum, New York, 1978).
14. Dillon, W. P. & Sougy, J. M. A. in *The Ocean Basins and Margins* Vol. 2 (eds Nairn, A. E. M. & Stehli, F. G.) 315-390 (Plenum, New York, 1974).
15. Klemme, H. D. *Bull. Am. Ass. petrol. Geol.* **42**, 477-512 (1958).
16. Hallam, A. *J. Geol.* **79**, 129-157 (1971).

17. Badham, J. P. N. *J. geol. Soc.* **139**, 495–504 (1982).
18. Sougy, G. *Bull. Soc. geol. Fr.* **11**, 133–149 (1969).
19. Schenk, P. E. in *North Atlantic Borderlands* (eds Kerr, J. W. & Ferguson, L.) 119 (Canadian Society of Petroleum Geologists, 1981).
20. Rona, P. E. *Bull. Am. Ass. petrol. Geol.* **54**, 129–157 (1970).
21. King, P. B. in *The Ocean Basins and Margins* Vol. 3 (eds Nairn, A. E. M. & Stehli, F. G.) 201–241 (Plenum, New York, 1975).
22. Bird, J. M. & Dewey, J. F. *Bull. geol. Soc. Am.* **81**, 1031–1060 (1970).
23. Gobbett, D. J. in *Atlas of Palaeobiogeography* (ed. Hallam, A.) 151–158 (Elsevier, Amsterdam, 1973).
24. Hallam, A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **35**, 1–44 (1981).
25. Westermann, G. E. G. in *The Ammonoidea* (eds House, M. R. & Senior, J. R.) 459–498 (Academic, London, 1980).
26. Johnson, J. G. & Boucot, A. J. in *Atlas of Palaeobiogeography* (ed. Hallam, A.) 89–96 (Elsevier, Amsterdam, 1973).
27. Chaloner, W. G. & Lacey, W. S. *Spec. Pap. Palaeont.* **12**, 271–289 (1973).
28. Ballance, P. F. & Reading, H. G. (eds) *Spec. Publ. int. Ass. Sedimentol.* **5** (1980).
29. Arthaud, F. & Matte, P. *Bull. geol. Soc. Am.* **88**, 1305–1320 (1980).
30. Dewey, J. F. *J. geol. Soc.* **139**, 371–412 (1982).
31. Ziegler, P. A. *Geological Atlas of Western and Central Europe* (Shell, London, 1982).
32. Ziegler, A. M. et al. in *Paleobotany, Paleocology and Evolution* (ed. Niklas, K. J.) 231–266 (Praeger, New York, 1981).

Desert palaeofloods in central Australia

Victor R. Baker, G. Pickup* & Henry A. Polach†

Department of Geosciences, The University of Arizona, Tucson, Arizona 85721, USA

* CSIRO Central Australian Laboratory, PO Box 2111, Alice Springs, NT, Australia 5750

† Radiocarbon Dating Research Laboratory, The Australian National University, PO Box 4, Canberra, ACT, Australia 2600

Hydrological conditions in arid regions are notoriously difficult to characterize in time and space. The inherent variability of rainfall and the resulting runoff poses problems for both economic development and palaeoenvironmental studies. Quantitative data on past floods are especially useful because the extreme rainfalls that generate desert floods are important environmental determinants in the world's arid environments. Unfortunately these are the environments that lack long-term hydrological records. We report here on the application of a palaeohydrological procedure^{1,2} that utilizes flood sediments as palaeostage indicators. By determining the ages of the responsible palaeofloods it is possible to evaluate their frequency of occurrence. The magnitude and frequency of floods on major desert stream or wadi systems provide critical palaeoclimatic information by delimiting areal and temporal shifts in rainfall-inducing weather systems. Central Australia is an ideal region in which to illustrate this technique because major phases of flooding correspond to the increased extratropical influence of the north Australia monsoons and/or tropical cyclones. We demonstrate here the use of palaeoflood hydrology to evaluate the recent phase of immense floods on the Finke River near Alice Springs in arid central Australia. The method approximates the magnitudes of the three great floods of 1967, 1972 and 1974, and it proves to be useful in other desert regions where conventional hydrological information is lacking.

The Finke River system (Fig. 1) heads in Archaean gneiss, granite and schist of the Arunta Block terrain about 75 km west of Alice Springs, Northern Territory, Australia. Deriving a sand and gravel load from the grussification of the Arunta rocks, the Finke transects a series of continuous east–west ridges and mountain ranges as it maintains its ephemeral course southward into the duricrusted plains and sand ridge terrains of northern South Australia to its terminus at Lake Eyre (elevation –16 m). Spectacular water gaps and gorges are developed through the resistant sandstone ridges of the Macdonnell, Krichauff and James Ranges. This discordance of drainage with regional structure has been attributed to superimposition³, antecedence⁴, inheritance⁵, and to complex combinations of inheritance, local superimposition and captures⁶. Because of very deep, slot-like fluvial entrenchment, relatively small discharge increases for the occasional Finke River floods produce relatively large increases in river stage for the canyons and gorges.

The entire Finke River system is ephemeral, with flows occurring only after periods of intense rain. The sand load of

the Finke is carried in suspension by turbulent, high-velocity flood flows through the gorge reaches. At the mouths of tributaries to a deep bedrock canyon, flood flow velocities are markedly reduced, and slow moving eddies may develop at peak discharges⁷. In such situations, suspended sand rapidly settles, comprising sequences of slack-water deposits which can be used for palaeoflood hydrologic interpretation^{1,2,7}.

Average annual rainfall decreases from 250 mm at Alice Springs to <125 mm in the south-east corner of the Northern Territory (Fig. 1). However, heavy rains are possible when the predominating high-pressure system of warm dry air is destabilized by influxes of moist air. The major flood season is in summer when tropical air penetrates from the north. Rains of 120–350 mm were induced by unusual monsoonal weather between 5 February and 8 March 1967 (ref. 8). The 1967 floods of the Finke River were considered the largest in memory⁸, or at least since 1895, when the Hermannsburg Mission recorded 240 mm in mid-January (Archivist, Lutheran Church of Australia, personal communication). The 1967 flows were surpassed by floods in March 1972 and January 1974. The 1974 floods are associated with the highest annual rainfall since recordings began in 1873 at Alice Springs Telegraph Station⁹.

At Hermannsburg, 115 km WSW of Alice Springs, the Finke River drains ~4,000 km² of predominantly sandy plains and some mountains. Downstream the river enters a gorge through the Krichauff Ranges (Fig. 2). In the constricted reach at the mouth of the gorge, maximum flow depths reached 5.5 m in 1967, and 6.0 m in 1972 and 1974. These stages were estimated in post-flood surveys by the Northern Territory of Australia, Department of Transport and Works, and they exceed by at least 2.5 m all other flow event stages that have been observed at the site. Discharge estimates for these desert floods cannot be satisfactorily achieved by extrapolating the Hermannsburg gauge record, which is limited to low flow events (all <10 m³ s⁻¹ achieved in flows of 1 m or less in depth). Such an extrapolation can only provide a crude guess of the flow magnitude, indicating that the three floods achieved ~2,000 m² s⁻¹. A post-flood survey of the Finke at Henbury (~100 km downstream of Hermannsburg) estimated the 1974 event as 2,350 m³ s⁻¹, achieving a mean flow velocity of 2.4 m s⁻¹ (P. Jolly, unpublished report to the Northern Territory of Australia, Department of Transport and Works, 1975).

We analysed slack-water sedimentary sequences at three sites along the Finke River gorge ~10 km downstream of Hermannsburg (Fig. 2). Site 2 illustrates the channel cross-section and the flood stratigraphical relationships (Fig. 3a). The flood slack-water deposits are well sorted fine sand with a graphic mean size¹⁰ of 0.10 mm and an inclusive graphic standard deviation¹⁰ of 0.7 mm. Three distinct flood-sedimentation units are present in pit 2 which correspond to at most three major floods capable of generating flow depths of 7 m or more in the adjacent channel of the Finke River (Fig. 3a). Extension of pit 2 to a depth of 1.75 m shows that the lowermost flood unit coarsens with depth and is at least 1.2 m thick. If the three units represent the 1967, 1972, and 1974 floods, the greater stages achieved at site 2 than at Hermannsburg result from a narrower channel in the gorge.

Radiocarbon analyses were performed on wood and charcoal from the slack-water sites. Two charcoal samples at site 2, pit 2 (Fig. 3a) yielded ultramodern dates ($\delta^{14}\text{C}$ of +26.1 ± 25.1‰ and $\delta^{14}\text{C}$ of +22.2 ± 14.8‰ for samples ANU-2400 and ANU-2402 respectively). 'Ultramodern' refers to radiocarbon ages in which the ¹⁴C activity is anomalously high because of the influence of extensive nuclear bomb testing in the early 1950s. We determined approximate dates for our samples by reference to the steeply rising Southern Hemisphere ¹⁴C concentration since 1955 (ref. 11 and J. Vogel, personal communication). By this procedure the samples 'date' as 1957. Sample ANU-2401 yielded a ¹⁴C age of 1,270 ± 380 yr BP ($\delta^{14}\text{C}$ of -146.7 ± 38.7‰). This charcoal was probably introduced by the reworking of older fluvial sediments, illustrating the problem of charcoal dates in alluvial sequences¹². An analysis of an associated

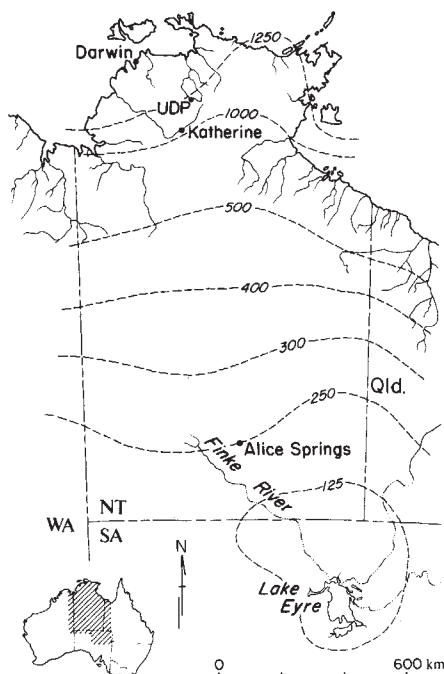


Fig. 1 Location map for Finke River, Northern Territory, Australia. The dashed lines show isohyets of mean annual precipitation in mm.

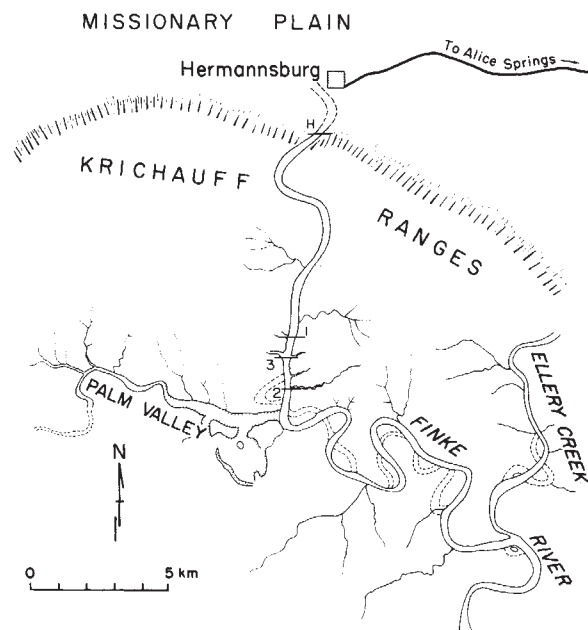
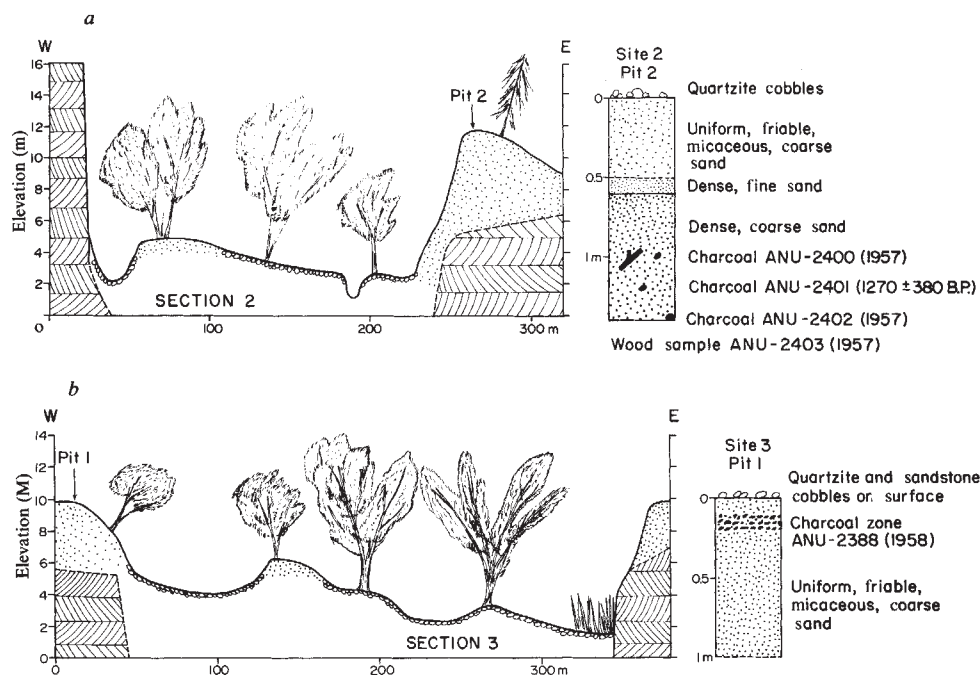


Fig. 2 Location map for cross-sections and study sites in the Finke River gorge (numbered). H, the Hermannsburg gauge site. An ancient system of bedrock palaeomeanders (dashed lines) forms a palimpsest with the modern drainage. Cross-sections 2 and 3 are shown in Fig. 3.

Fig. 3 Surveyed cross-section of the Finke River at: *a*, study site 2, showing the location and stratigraphy of flood slack-water deposition exposed in pit 2; *b*, study site 3, showing development of flood-transported charcoal zone in the slack-water sediments exposed in pit 1. The cross-section location is shown in Fig. 2.



wood fragment (sample ANU-2403 with $\delta^{14}\text{C}$ of $-2.2 \pm 5.7\%$) confirmed the 1957 date, as did an analysis from site 3, where the upper flood deposit contained a prominent charcoal layer (Fig. 3b). Correlation of the site 3 flood layer is established by the ultramodern date ($\delta^{14}\text{C}$ of $+97.8 \pm 16.1\%$ or 1958 by the Polach procedure¹¹).

The radiocarbon analyses confirm that the three floods represented at site 2 (Fig. 3a) are the 1967, 1972 and 1974 events, as those are the only post-1957 floods capable of inundating the site. Moreover, the heights of the flood deposits permit an estimate of the flood stage responsible for deposition. By surveying the three cross-sections we found the highest slack-water deposition to exhibit a fall of 2.1 m over the 1,900 m reach between sections 1 and 2 (Fig. 2). Using this gradient, the

existing channel geometry, and assuming a roughness coefficient of 0.04 and an energy coefficient of 1.5, we calculated by the slope-area hydraulic procedure¹³ a discharge of $3,360 \text{ m}^3 \text{ s}^{-1}$ and a mean flow velocity of $2.05\text{--}2.5 \text{ m s}^{-1}$. Thus, the flood sedimentation record provides an independent means of analysing past floods in the region.

Palaeoflood estimates from slack-water sequences are subject to several qualifying assumptions and error sources¹⁴. Both the roughness and energy coefficients must be estimated, and the slope of the slack-water deposit surfaces need not correspond to the actual water surface during the flood. Moreover any scour of the Finke River bed after the slack-water deposition would result in overestimation of the palaeoflood peak, while aggradation of the bed would result in underestimation. Scoured

bases around large red gum trees on the canyon floor and armoured pavements of cobbles in scour channels (Fig. 3) indicate that the former is the case for this analysis. Despite such reservations, palaeoflood flow approximations have proved to be exceedingly useful in data-sparse arid regions subject to large rare floods and which lack independent sources of flood data^{1,15-18}.

Received 28 July; accepted 26 November 1982.

1. Baker, V. R., Kochel, R. C. & Patton, P. C. in *The Hydrology of Areas of Low Precipitation*, 3-9 (International Association of Hydrological Sciences, Publ. No. 128, 1979).
2. Kochel, R. C. & Baker, V. R. *Science* **215**, 353-361 (1982).
3. Ward, L. K. *Trans. R. Soc. S. Aust.* **49**, 61-85 (1925).
4. Madigan, C. T. *Geogr. J.* **78**, 417-431 (1931).
5. King, L. C. *Proc. R. Soc. Vict.* **62**, 79-95 (1950).
6. Mabbitt, J. A. in *Essays in Geomorphology* (ed. Dury, G. H.) 83-119 (Heinemann, New York, 1966).
7. Patton, P. C., Baker, V. R. & Kochel, R. C. in *Adjustments of the Fluvial System* (eds Rhodes, D. C. & Williams, G.) 225-252 (Allen & Unwin, London, 1979).
8. Williams, G. E. *J. Hydrol.* **11**, 185-200 (1970).

We thank P. Russell, T. J. Verhoeven, and G. J. Woolfrey of the Northern Territory of Australia, Department of Transport and Works, Alice Springs, for assistance with field work, supporting data, and arrangements for this study. V.R.B.'s participation in the project was made possible by a Senior Fulbright Scholar award at Australian National University, administered by the Australian-American Educational Foundation.

9. Verhoeven, T. J. *Inst. of Engineers, Australia, Nat. Conf. Publ. No.* 7715, 30-34 (1977).
10. Folk, R. L. *Petrology of Sedimentary Rocks* (Hemphill's, Austin, 1974).
11. Polach, H. *Aust. National Univ. Radiocarbon Lab. Res. Rep.* 25-26 (1979).
12. Blong, R. J. & Gillespie, R. *Nature* **271**, 739-741 (1978).
13. Chow, V. T. *Open-Channel Hydraulics* (McGraw-Hill, New York, 1959).
14. Baker, V. R., Kochel, R. C., Patton, P. C. & Pickup, G. in *Modern and Ancient Fluvial Systems: Sedimentology and Processes* (International Association of Sedimentologists, in the press).
15. Baker, V. R. in *Scientific Basis of Water Resource Management* (National Academy, Washington, 1982).
16. Baker, V. R., Pickup, G. & Russell, P. *Geol. Soc. Am. Abstr. Prog.* **12**, 382 (1980).
17. Patton, P. C. & Dibble, D. S. *Am. J. Sci.* **282**, 97-121 (1982).
18. Kochel, R. C., Baker, V. R. & Patton, P. C. *Wat. Resour. Res.* **18**, 1165-1183 (1982).

Isomerization and aromatization of hydrocarbons in stretched sedimentary basins

Dan McKenzie*, Andrew S. Mackenzie†‡, J. R. Maxwell‡ & Cs. Sajgó§

* Bullard Laboratories, Department of Earth Sciences, University of Cambridge, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, UK

‡ Organic Geochemistry Unit, University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS, UK
§ M.T.A. Geokémiai Kutató Lab., Budapest XI Budaörsi út 45, H-1112, Hungary.

We have calculated the progress of two reactions, both involving biological marker compounds, which occur in shales rich in organic matter. Both reactions are associated with the early stages of oil generation. One of these reactions is an isomerization reaction of a sterane^{1,2}, the other involves the aromatization of monoaromatic steroid hydrocarbons^{3,4}. Both reactions have recently been discussed by Mackenzie *et al.*⁵. We demonstrate that the observed progress of these reactions within two basins formed by extension, the North Sea and the Pannonian Basin, can be reproduced in some detail by a simple model of basin formation⁶. We used samples from basins formed by stretching the lithosphere because both the subsidence history and the variation of heat flux with time is principally controlled by the amount of extension⁶⁻¹⁰. In contrast the subsidence of the other major class of sedimentary basins, which are formed by flexure of the continental lithosphere¹¹⁻¹³, can only be modelled when the geological history of the region is known in detail¹³, and such models do not constrain the history of the heat flux.

The two reactions we have studied are shown in Fig. 1. Both the 20R sterane and the monoaromatic steroid hydrocarbon are produced from biological precursors during early diagenesis of the sediment^{14,15}. The precursory reactions, involving the conversion of the sterols to steroid hydrocarbons, are essentially complete before the beginning of the reactions with which we are concerned. The first reaction involved the isomerization of the sterane with the 20R configuration, which alone is present initially, to a mixture of 20R and 20S steranes. Though the reaction mechanism is not yet known, such a reaction presumably results from the removal of the hydrogen attached to C-20, followed by its replacement. The other reaction involves the

aromatization of the monoaromatic steroid hydrocarbon (Fig. 1b) by the removal of a methyl group and several hydrogens. Unlike the isomerization reaction, the aromatization reaction is assumed to be irreversible and to convert all the monoaromatic, M, triaromatic, T, steroid hydrocarbons. The mechanism of this reaction is also unknown.

We have assumed that the progress of both reactions could be described by unimolecular first-order reaction laws of the form

$$\begin{aligned}\frac{dB}{dt} &= k_1C - k_2B \\ \frac{dC}{dt} &= k_2B - k_1C\end{aligned}\quad (1)$$

where B and C are the concentrations of the product (20S, T) and reactant (20R, M) respectively, and k_1 and k_2 are the reaction rates of the forward and backward reactions. We also assumed that $k_1 = 1.174 k_2$ for the isomerization reaction to give an equilibrium ratio of $[20S]/[20R]$ in agreement with that proposed by van Grass *et al.*¹⁶, and that $k_2 = 0$ for the aromatization reaction. We used Arrhenius's law to relate the reaction rates to the temperature

$$k = A \exp(-E/RT) \quad (2)$$

where A , the frequency factor, and E , the activation energy, were taken to be constant, and R is the gas constant and T the absolute temperature. The stretching model of a sedimentary basin provides the temperature history¹⁰ $T(t)$. Hence it is straightforward to integrate equation (1) numerically for any choice of A and E to obtain $C(t)$. A convenient comparison of the observations with the calculations is obtained by plotting the progress of the isomerization reaction measured by

$$y = \frac{[20S]}{[20S] + [20R]} \quad (3)$$

against that of the aromatization reaction

$$x = \frac{[T]}{[M] + [T]} \quad (4)$$

The values of x and y of a sample can be directly determined by using results obtained by gas chromatography/mass spectrometry and hence the position of a point corresponding to x and y is independent of any basin model. Integration of equation (1) for both reactions generates a curve in the x, y plane with which the observations can be compared. We will refer to such plots as AI diagrams. To carry out such calculations we need estimates of six parameters: the amount of extension, β , defined as the ratio of the final to the initial area of the part of the basin with which we are concerned, the time since the

† Present address: Geochemistry Branch, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN, UK.

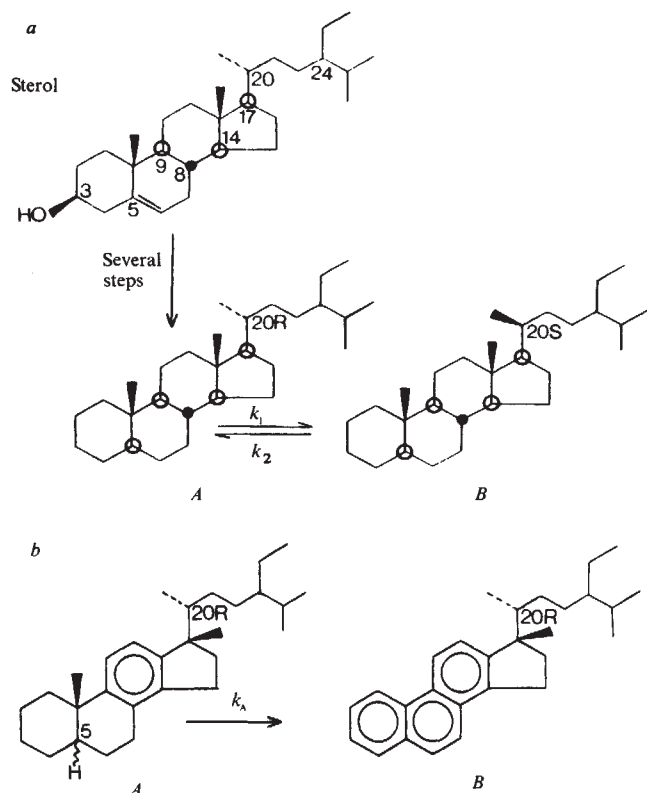


Fig. 1 The two reactions we used are: *a*, the isomerization of 20R sterane to an approximately equal mixture of 20R and 20S forms; and *b*, the aromatization of two monoaromatic steroid hydrocarbons, presumed to be isomeric at C-5. Bonds between carbons are shown as straight lines, and the atoms are at the junctions. Bonds not shown are attached to hydrogens. An open circle or dotted line (solid circle or heavy line) indicates the carbon or the bond is below (above) the paper. A circle inscribed in a hexagon indicates an aromatic ring.

stretching event, and the activation energies and frequency factors of the two reactions. Estimates of the first two parameters have been made from studies of the geology of the North Sea⁷ and Pannonian Basin⁹. To estimate trial values of A and E for the two reactions we integrated equation (1) analytically, taking k_1 and k_2 to be constant, and then used the stretching model to estimate the time that the samples spent within 15 °C of their present temperature¹⁷. In both cases this time was about $0.4 t_s$, where t_s is the time which has elapsed since the stretching event. Estimates of A and E were then made by plotting $\log_e k$, determined from x and y , against the reciprocal of the present temperature. These trial values of A and E were then used to integrate equation (1) numerically, using the temperature history obtained from the sedimentary basin model, to generate a curve in the AI diagram. The values of A and E were then adjusted until the calculated curves matched the observations.

The results of these calculations are compared in Fig. 2 with the positions and depths of several shale samples from the northern part of the North Sea. The shape of the calculated curves does not depend strongly on β , and is principally controlled by the ratio of the two reaction rates. The locations of the calculated depth marks every 200 m along the curves are, however, governed by the rate of each reaction, rather than by the ratio of reaction rates. Hence it is possible to fit the x , y positions of the observations with a variety of values of A and E , but much of this ambiguity can be removed by matching the sample depths with those calculated, as well as with the shape of the curves. Although the observations are scattered, probably because the thermal evolution of individual samples is somewhat more complicated than that of the model basin, their x , y locations and the depths agree reasonably with the calculations.

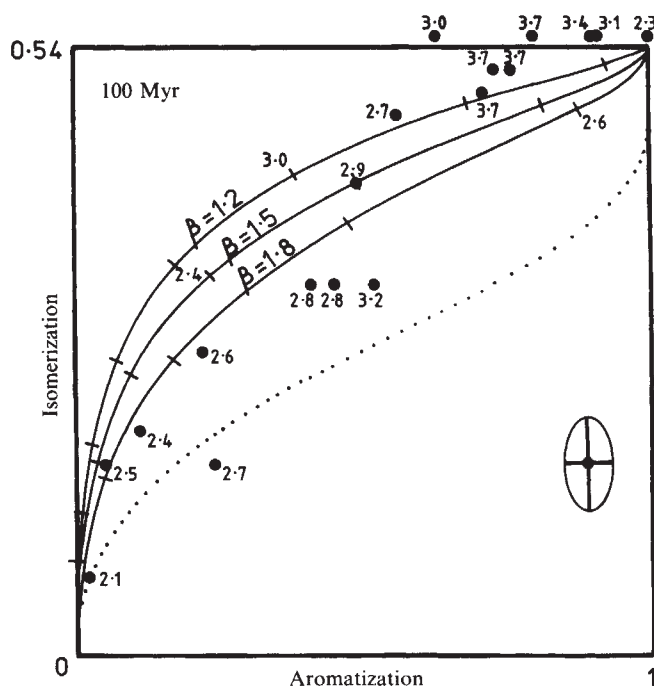


Fig. 2 An AI diagram for the North Sea. The curves are obtained from model calculations, and show the behaviour 100 Myr after extension by the amount indicated by the value of β . The short cross lines show the depth in km, and are at 200-m intervals. The continuous curves were obtained using the values of A and E given in the text for the North Sea, the dotted line using those for the Pannonian Basin and $\beta = 1.5$. The solid dots show the x , y values obtained from several conventional cores in the northern part of the North Sea, marked with their depths below the sea floor in km. The ellipse indicates the greatest error we believe occurs in the measurement of x and y . The standard deviation of the observations is probably smaller.

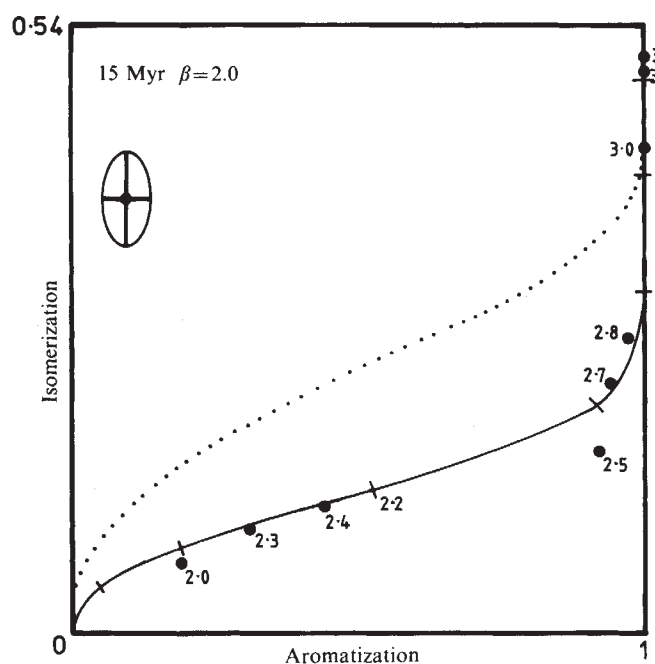


Fig. 3 An AI diagram for a single hole in the south-east part of the Pannonian Basin. The dotted line was obtained using the values of A and E for the North Sea the solid line with those for this basin. The extension was by a factor of 2 15 Myr ago for both curves (see Fig. 2. for details).

The AI diagram for the Pannonian Basin (Fig. 3) shows less scatter, probably because all samples were obtained from a single hole. Unlike the North Sea, the aromatization reaction has occurred faster than the isomerization reaction. We believe that this is the case because the subsidence rate, and hence the heating rate, is more rapid in the Pannonian Basin. The agreement between the calculated curve and the observations is excellent.

The activation energies used to obtain the curves in Figs 2 and 3 were 200 kJ mol^{-1} for aromatization and 91 kJ mol^{-1} for isomerization. However, we did not succeed in fitting the observations with the same values of the frequency factors for both basins. For the North Sea we used 10^{-2} s^{-1} and 10^{14} s^{-1} for isomerization and aromatization respectively, whereas for the Pannonian we used $6 \times 10^{-3} \text{ s}^{-1}$ and $1.8 \times 10^{-4} \text{ s}^{-1}$ for the same two reactions. It is not yet clear whether these differences are the result of real differences in the reaction rates, which could for instance be produced by catalytic effects, or whether they result from oversimplifications in the model calculations, especially in the case of the Pannonian Basin⁹. The frequency factor for the isomerization reaction is surprisingly low, and if confirmed will provide information about the reaction mechanism.

It is perhaps surprising that we have estimated the frequency factors and activation energies for these two reactions using a model for the evolution of a sedimentary basin, rather than from the results of laboratory experiments. The problem is that, if our estimates of A and E are correct, the temperature must be increased to more than 200°C and 600°C before measurable aromatization and isomerization respectively occur over a period of several months. As the temperatures in the shale samples from the basins have always been less than 140°C , there must be some doubt whether the same values of A and E will apply in the laboratory and in the basins. It is, however, of interest that the aromatization constants obtained from geological observations are consistent with the results of laboratory experiments between 200°C and 285°C lasting up to 90 days (ref. 18). In the same experiments there was no significant isomerization, a result which is also consistent with our estimate of the isomerization rate.

This investigation indicates that small quantities of biological markers (commonly $<10^{-12} \text{ kg}$) contain a record of the subsidence history of a sedimentary basin. The progress of the reactions is especially sensitive to uplift, which can freeze both of those discussed here if the uplift is sufficiently great. A more detailed account of these investigations will be published elsewhere¹⁹. This is Department of Earth Sciences contribution no. 258.

Expulsion of petroleum hydrocarbons from shale source rocks

A. S. Mackenzie*, D. Leythaeuser & R. G. Schaefer

Institute of Petroleum and Organic Geochemistry (ICH-5), KFA-Jülich, PO Box 1913, D-5170 Jülich 1, FRG

M. Bjorøy

Continental Shelf Institute (IKU), Hakon Magnussons gt. 1B, Trondheim, Norway

There is compelling evidence that movement of petroleum from organic-rich shales into sandstones and limestones, occurs in the subsurface¹. This expulsion of material must occur if crude oil accumulations are to form, and it is termed primary migration². Earlier studies^{3,4} suggested that this movement occurs with chemical fractionation: some components are more mobile than others. One of the agents of expulsion at early maturity stages is compaction, which is not only capable of forcing water, but also organic components out of the pore network of a source rock^{2,5,6}. Because the fractionation effects associated with expulsion provide clues to the transport mechanisms involved, we have made detailed quantitative comparisons of the hydrocarbons of shales of different thicknesses, the centre and edges of thick shales and sandstones adjacent to shales from two cored, interbedded shale and sandstone sequences from Spitsbergen Island, Svalbard, Norway. These observations confirm considerable movement of hydrocarbons in the subsurface, with lower molecular weight components being more mobile. Up to 90% the original amount of n -pentadecane may be lost from very thin shales sandwiched between sandstone horizons and from shales close to a lithological boundary with sandstone.

Failure to understand petroleum migration is partly the result of insufficient observations of petroleum moving from one place to another⁷. The expected concentration levels involved in primary migration will be small and therefore only geochemical analyses are likely to detect such movement. The two sequences chosen were cored continuously to 142 and 239 m at Reindalen and Adventdalen, respectively, on Spitsbergen Island in areas of continuous permafrost. The Reindalen sequence was Palaeocene in age, and the Adventdalen strata were Lower Cretaceous. Minor accumulations of gas were encountered in both wells. Rock-Eval pyrolysis⁸ suggested all samples contained type III kerogens^{9,10}, and the vitrinite reflectance of coals that occur in both sequences was 0.82% at Reindalen and 0.85% at Adventdalen. This maturity is normally associated, for type III organic matter, with the early stage of oil generation⁶. Studies of C_2 – C_8 hydrocarbons in the Reindalen core have revealed major redistribution of material in this range¹¹.

The powdered core samples were extracted and the resulting extracts fractionated by methods described previously^{12,13}. The alkanes were quantitated by comparison of the gas chromatography analysis of a known amount of n -pentadecane as external standard. The alkane fractions were analysed by gas chromatography–mass spectrometry (GC–MS)¹⁴ to determine the sterane and triterpane distributions. Because the evidence suggests that no major migration has occurred in the sedimentary column examined, for components $>\text{C}_{25}$, the patterns of the higher molecular weight steranes and triterpanes can be used to refine the assignment of organic matter type. Differences which have resulted from migration are then assessed by comparing hydrocarbon concentration levels of samples which contain a similar type of organic matter. The details of the sterane and triterpane classification will be reported elsewhere¹⁰. They could be used to subdivide the type III

Received 14 September; accepted 6 December 1982.

1. Mackenzie, A. S., Patience, R. L., Maxwell, J. R., Vandenbroucke, M. & Durand, B. *Geochim. cosmochim. Acta* **44**, 1709–1721 (1980).
2. Seifert, W. K. & Moldovan, J. M. *Geochim. cosmochim. Acta* **42**, 77–95 (1978).
3. Mackenzie, A. S., Hoffman, C. F. & Maxwell, J. R. *Geochim. cosmochim. Acta* **45**, 1345–1355 (1981).
4. Ludwig, B., Hussler, G., Wehrung, P. & Albrecht, P. *Tetrahedron Lett.* **22**, 3313–3316 (1981).
5. Mackenzie, A. S., Lamb, N. A. & Maxwell, J. R. *Nature* **295**, 223–226 (1982).
6. McKenzie, D. P. *Earth planet. Sci. Lett.* **40**, 25–32 (1978).
7. Slater, J. G. & Christie, P. A. F. *J. geophys. Res.* **85**, 3711–3739 (1980).
8. Royden, L., Slater, J. G. & Von Herzen, R. P. *Bull. Am. Ass. petrol. Geol.* **64**, 173–187 (1980).
9. Slater, J. G. *et al. Earth planet. Sci. Lett.* **51**, 139–162 (1980).
10. McKenzie, D. P. *Earth planet. Sci. Lett.* **55**, 87–98 (1981).
11. Gunn, R. *Geophysics* **12**, 238–255 (1947).
12. Heiskanen, W. A. & Vening Meinesz, F. A. *The Earth and its Gravity Field* (McGraw Hill, New York, 1958).
13. Beaumont, C. *Geophys. J. R. astr. Soc.* **65**, 291–329 (1981).
14. Mackenzie, A. S., Brassell, S. C., Eglinton, G. & Maxwell, J. R. *Science* **217**, 491–504 (1982).
15. Ludwig, B., Hussler, G., Wehrung, P. & Albrecht, P. in *Advances in Organic Geochemistry 1981* (eds Bjorøy, M. *et al.*) (Wiley, Chichester, in the press).
16. van Grass, G., Baas, J. M. A., van de Graaf, B. & de Leeuw, J. W. *Geochim. cosmochim. Acta* **46**, 2399–2402 (1982).
17. Hood, A., Gutjahr, C. C. M. & Heacock, R. L. *Bull. Am. Ass. petrol. Geol.* **59**, 986–996 (1975).
18. Mackenzie, A. S., Lewis, C. A. & Maxwell, J. R. *Geochim. cosmochim. Acta* **45**, 2369–2375 (1981).
19. Mackenzie, A. S. & McKenzie, D. P. *Geol. Mag.* (in the press).

* Present address: BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN, UK.

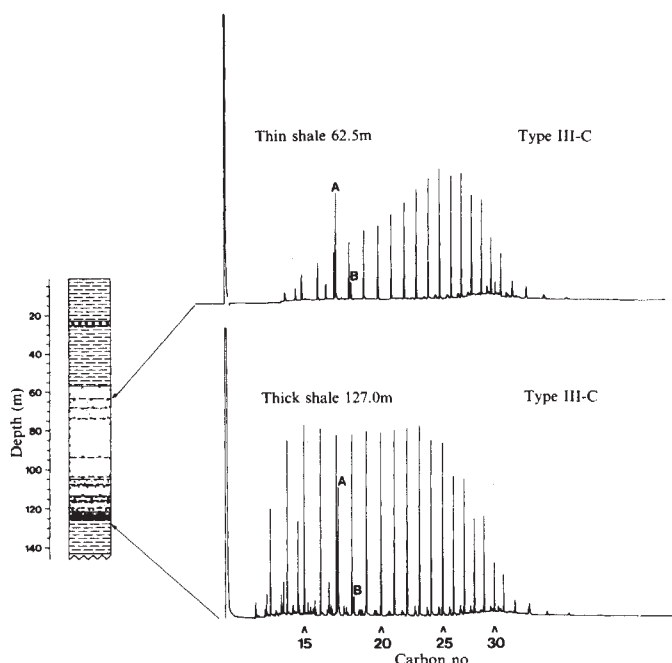


Fig. 1 Comparison of C_{15+} alkane gas chromatograms from a thin (5 cm) shale and the centre of a thick shale unit from the Reindalen core, Spitsbergen. The positions of the two sample sites are arrowed on the lithologic column of the core.

designation derived from the Rock-Eval analyses into six subgroups (III-A to III-F).

Geochemical evidence of the preferential migration of C_{15} – C_{19} n -alkanes, relative to other components, into a conglomerate in the Reindalen sequence and from the underlying and overlying siltstones, has been presented elsewhere¹⁵. To investigate this fractionation further, the alkane distributions from thin shales have been compared with those from the centre of thicker horizons of the same lithology and designated organic matter type. It is believed that thin shales have a better chance of achieving compaction equilibrium, because the expelled fluids can escape in this case, into the surrounding sandstones. Less material is expected to be expelled from the centres of thicker shales, as the pore fluids will escape less easily, resulting in undercompaction and abnormal pressures². Figure 1 compares the alkane distributions, as revealed by gas chromatography, from a thin shale (5 cm) in the middle of a sandstone body with those of a sample from the centre of a thick shale unit. The thin shale appears to have lost some of its lower molecular weight n -alkanes relative to the thick shale, with pristane and phytane appearing less affected. We refer to the thick shale distribution as 'unmodified' and to that of the thin shale as 'depleted'. This does not mean that no loss of hydrocarbons has occurred from the thick shale unit. Although no porosity measurements were available, we suspect the thick shale unit has experienced less compaction and expulsion than the thin shale, and so has a better chance of having preserved the original indigenous distribution.

Since the depleted thin shale and the unmodified thick shale (Fig. 1) have similar amounts of organic carbon (4.8 and 5.7% respectively) the amounts of alkanes were used to calculate expulsion efficiencies for individual hydrocarbons from the expression.

$$\frac{\text{unmodified} - \text{depleted}}{\text{unmodified}} \times 100$$

Expulsion efficiency decreases with increasing carbon number, so that nearly 90% expulsion is calculated for C_{15} , but no significant expulsion is suspected beyond C_{25} (Fig. 2). The estimated expulsion efficiencies of pristane and phytane are about a half of the values for n -alkanes of the same carbon

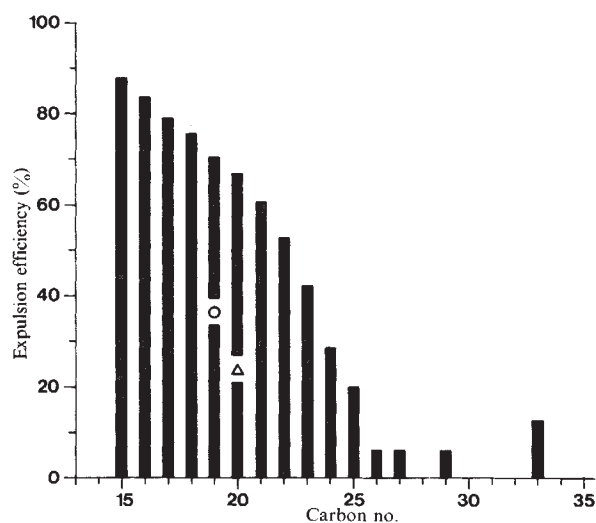


Fig. 2 Expulsion efficiencies of normal and isoprenoid alkanes as a function of carbon number for a thin (5 cm) source rock shale unit from the Reindalen corehole, Spitsbergen. Expulsion efficiencies were obtained by a quantitative comparison of the alkane distributions of two shale samples shown in Fig. 1.

number (Fig. 2). In sharp contrast, the expulsion efficiency of the total solvent soluble organic matter (consisting of aliphatic and aromatic hydrocarbons and heterocompounds) was calculated as 10%, so that lower carbon number n -alkanes seem to be expelled more easily than the rest of the soluble organic matter present. There was no preferential expulsion of total alkanes relative to total aromatic hydrocarbons observed.

The results for two examples from the Adventdalen core are also explicable by redistribution with the associated fractionation effects. In Fig. 3, a sandstone interval within a siltstone sequence is considered. Towards the siltstone–sandstone contact, below a depth of 38 m, a siltstone sample whose C_{15} – C_{19} n -alkanes compared with the three samples above 38 m depth were present in lower relative abundances, was encountered (Fig. 3). The sandstone sample has a unimodal distribution, centred on n -heptadecane. These patterns would be expected if the siltstone adjacent to the sandstone had been depleted in hydrocarbons, by the movement of material into the sandstone, which is enriched. If the lower carbon number n -alkanes migrate more readily, the variations in n -alkane patterns seen in Fig. 3 would be predicted. Careful examination of the sterane and triterpane patterns of the samples shown by arrows in Fig. 3 revealed no significant differences. The organic matter type was called III-F (III-A to III-C occur in the Reindalen core; III-D to III-F in the Adventdalen samples).

Figure 4 considers a 7-m thick shale bed within a sandstone body. Four samples from the middle of the shale gave unmodified patterns. The two samples nearest the top and bottom shale–sandstone contacts (shown by open arrows in Fig. 4) have depleted n -alkane distributions: the lower molecular weight n -alkanes are present in smaller relative amounts than in the middle part of the shale. The sandstone samples adjacent to the contacts have enriched n -alkane patterns, with a bias towards lower carbon numbers. The results in Fig. 4 could be accounted for by the redistribution of material at the top and bottom of the shale, into the overlying and underlying sandstone beds respectively, with chemical fractionation.

The phenomena illustrated in Figs 1–4 are unlikely to have arisen from variable extents of biodegradation, which would remove lower molecular weight n -alkanes preferentially. If biodegradation had occurred, then it would be expected to be

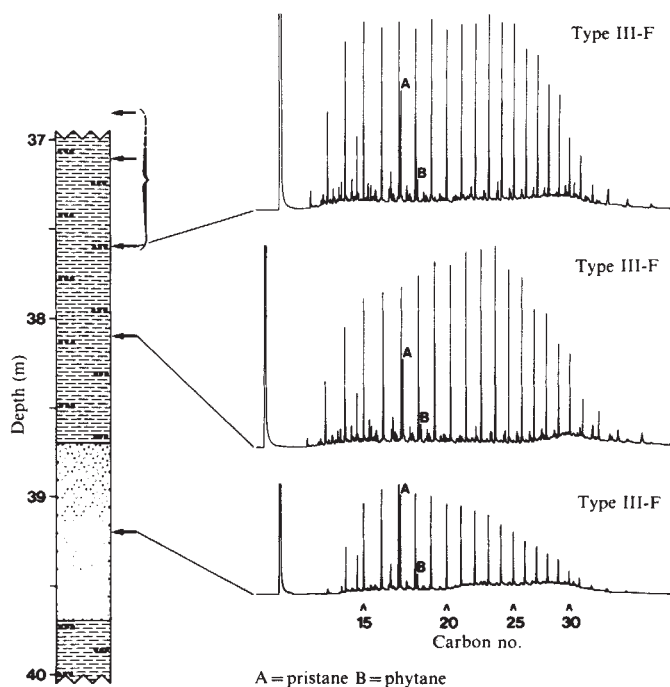


Fig. 3 Examination of the effects of expulsion of C_{15+} alkanes from a shale unit into a thin (1 m) interbedded sandstone layer, in the Adventdalen sequence, Spitsbergen, on the alkane compositions. The original bimodal alkane distribution, thought to be represented by the centre of the upper continuous shale unit, is changed into a unimodal, heavy-end biased distribution in the shale sample closest to the contact with the underlying sandstone unit. The sandstone has a unimodal light-end biased alkane distribution. The samples considered are shown as arrows.

most advanced in the more permeable layers (the sandstones), where a higher flow of nutrient- and oxygen-rich water would be predicted. None of the sandstone samples examined showed evidence of depletion of lower molecular weight n -alkanes.

Geochemists have previously tried to explain most variations in alkane distributions from source rocks in terms of maturity and organic matter type¹⁶. The present study suggests the effects of migration must also be considered. The results suggest that thin shales and the edges of thicker shale units have been more depleted in C_{15+} solvent soluble material than the centres of thicker shales in shale/sandstone sequences, and that the lower molecular weight n -alkanes ($<C_{19}$) are preferentially expelled. Some quantitative data have been presented, which suggest that up to 90% of lower carbon number n -alkanes may be expelled in favourable conditions, but in such cases only up to 10% of the total extract is expelled. It is not wise to overemphasize such data because this study is necessarily restricted to one-dimensional movement (up and down the hole). Samples with n -alkanes showing an enriched compositional pattern need not show quantitative enrichment, because the main mass of migrating fluids could have continued further, in, for example, a lateral direction. Likewise, shale samples with a depleted pattern of n -alkanes may have subsequently been replenished by a migration within the shale associated with less chemical fractionation.

The complexities are great because so many factors play a part, and it is unlikely the trends reported here would have been observed without the thorough geochemical examination of over 150 samples from just 320 m of core. Further studies of this nature are required, because the extent to which organic components can be expelled from an organic-rich shale partly determines its potential as a source rock for oil. In the past the potential of a source rock has been judged by the amount and composition of extractable material retained⁶, but 'good quality' source rocks, by definition, must also be capable of losing a significant proportion of the hydrocarbons generated. Quantita-

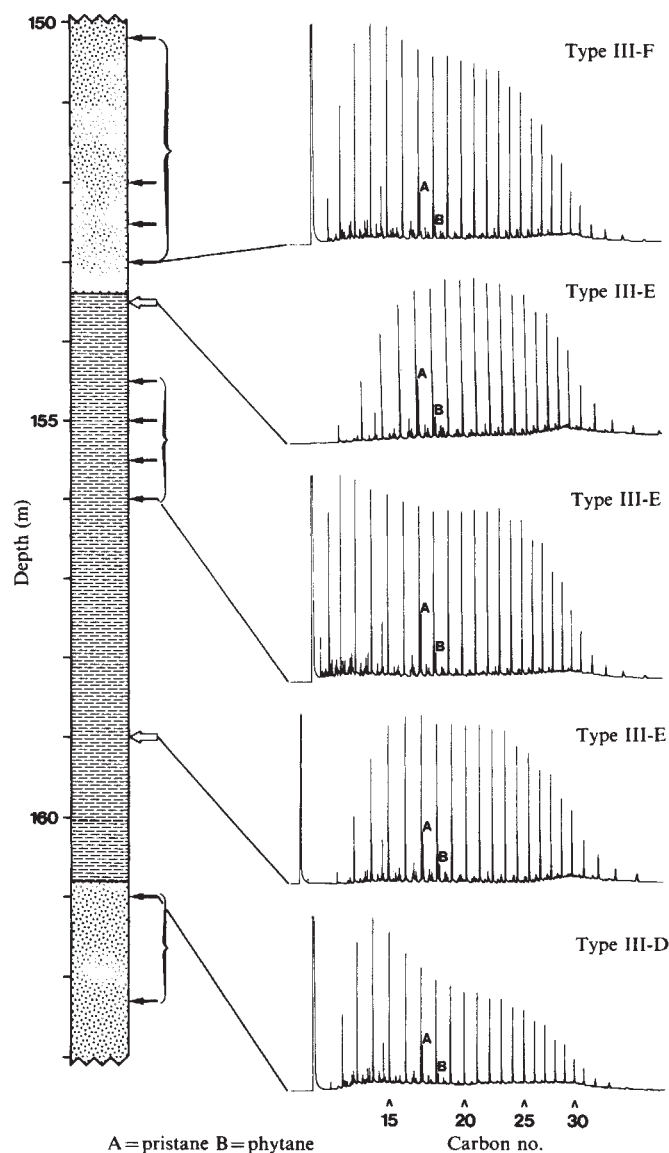


Fig. 4 Examination of the effects of expulsion of C_{15+} alkanes from a 7 m shale unit and into the underlying and overlying sandstone units in the Adventdalen sequence, Spitsbergen, on the alkane compositions. The original bimodal alkane distribution, thought to be represented by the shale samples at around 155 m, becomes a unimodal, heavy-end biased distribution in the two samples (shown as open arrows) nearest to the contacts with the overlying and underlying sandstones. The sandstones have unimodal light-end biased alkane distributions. The samples considered are shown as arrows.

tive evaluation of the timing and extent of expulsion is important in petroleum exploration and this should improve when the mechanisms involved are understood better.

The expected degree of compaction of a shale sample appears, at the maturity stage of the samples considered, to control the extent to which its soluble organic matter content has been expelled. It would be predicted that thin shales and the edges of thicker shales in shale/sandstone sequences will be more compacted than the centres of thicker shales, thus explaining the greater hydrocarbon depletion of the former. An alternative explanation could, however, be that because thin shales and the margins of thick shales are closer to more porous sandstones than the centres of thick shale units, expulsion by the release of the increased pressure built up in organic-rich shales during oil generation by the thermal breakdown of kerogen may have been easier⁶. The apparent enrichment of

lower carbon number *n*-alkanes in migrated mixtures is probably due to their lower tendency to be absorbed or adsorbed by the matrix of the host sediment relative to higher carbon number *n*-alkanes and the other solvent soluble components present^{17,18}. If this absorption-adsorption process is not easily reversed then the preferential retention of certain components could alone explain all of the effects reported above. If, however, desorption readily occurs, then the distributions must be the consequence of chromatographic effects, with, for

example, the higher molecular weight species moving more slowly. The increased viscosities resulting from the temperature drop associated with either the introduction of permafrost¹⁹ or uplift the sequence in the late Tertiary²⁰ may have slowed the migration sufficiently so as to help preserve any chromatographic separations.

We thank the Store Norske Spitsbergen Kulkompani, Longyearbyen, for the samples and A.S.M. thanks the Humboldt foundation for a fellowship.

Received 4 October; accepted 6 December 1982.

- Magara, K. *Bull. Am. Ass. petrol. Geol.* **64**, 2108–2117 (1980).
- Hunt, J. M. *Petroleum Geochemistry and Geology* (Freeman, San Francisco, 1979).
- Vandenbroucke, M. in *Advances in Organic Geochemistry 1971* (eds von Gaertner, H. R. & Wehner, H.) 547–565 (Pergamon, Oxford, 1972).
- Connan, J. & Cassou, A. M. *Geochim. cosmochim. Acta* **44**, 1–23 (1980).
- Magara, K. *Bull. Am. Ass. petrol. Geol.* **60**, 543–553 (1976).
- Tissot, B. & Welte, D. H. *Petroleum Formation and Occurrence* (Springer, Berlin, 1978).
- Roberts, W. H. & Cordell, R. J. *Introduction to Problems of Petroleum Migration* (eds Roberts, W. H. & Cordell, R. J.) (AAPG Series in Geology No. 10, 1980).
- Espitalié, J. *et al. Rev. Inst. fr. Petrol.* **32**, 23–42 (1977).
- Durand, B. & Espitalié, J. *C.R. hebdom. Séanc. Acad. Sci., Paris* **276**, 2253–2256 (1973).

- Leythaeuser, D. *et al. Bull. Am. Ass. petrol. Geol.* (submitted).
- Leythaeuser, D., Schaefer, R. G. & Pooch, H. *Bull. Am. Ass. petrol. Geol.* (in the press).
- Radke, M., Sittardt, H. G. & Welte, D. H. *Analyt. Chem.* **50**, 663–665 (1978).
- Radke, M., Willsch, H. & Welte, D. H. *Analyt. Chem.* **52**, 407–411 (1980).
- Rullkötter, J. & Wendisch, D. *Geochim. cosmochim. Acta* **46**, 1545–1554 (1982).
- Leythaeuser, D. *et al. in Advances in Organic Geochemistry 1981* (eds Bjorøy, M., *et al.*) (Wiley, Chichester, in the press).
- Tissot, B., Pelet, R., Roucagé, J. & Combaz, A. in *Advances in Organic Geochemistry 1975* (eds Campos, R. & Goni, J.) 117–154 (ENADIMSA, Madrid, 1977).
- Young, A. & McIver, R. D. *Bull. Am. Ass. petrol. Geol.* **61**, 1407–1436 (1977).
- Espitalié, J., Madec, M. & Tissot, B. *Bull. Am. Ass. petrol. Geol.* **64**, 59–66 (1980).
- Black, R. F. *Bull. geol. Soc. Am.* **65**, 839–856 (1954).
- Manum, S. B. & Throndsen, T. *Norsk Polarinstitt Arbok* 1977, 159–177 (1978).

New Palaeogene primate basicrania and the definition of the order Primates

R. D. E. MacPhee*, Matt Cartmill*†
& Philip D. Gingerich‡

* Department of Anatomy and † Department of Anthropology,
Duke University, North Carolina 27710, USA

‡ Museum of Paleontology, University of Michigan, Ann Arbor,
Michigan 48109, USA.

The anatomy of the posterior basicranium has been repeatedly invoked in systematic definitions of Primates. One widely cited definition^{1,2} of the order claims that 'all undoubted primates'³ are distinguished from other mammals by two basicranial specializations: (1) absence of a major vascular foramen on the medial side of the auditory region, and (2) development of the auditory bulla from the petrosal bone. As we show here, specialization (1) does not apply to the paromomyid *Ignacius*, and is of uncertain incidence in other unquestioned members of suborder Plesiadapiformes (archaic primates from the early Cenozoic of Europe and North America). Specialization (2) cannot be demonstrated without ontogenetic evidence, and all relevant plesiadapiform fossils are adult. In fact, the only plesiadapiform with an arterial pattern remotely resembling that of early primates of modern aspect (or 'euprimates') is the microsyopid *Cynodontomys*, but it is often regarded as non-primate because it lacks a petrosal bulla. Although plesiadapiforms resemble euprimates in traits of the cheek teeth and postcranium^{5–7}, some other (presumably non-primate) groups possess these traits as well. Since the order Primates is not clearly definable by unique specializations, the best grounds for regarding plesiadapiforms as euprimate antecedents are stratigraphic and phenetic. This fact may be best expressed by systematic arrangements that emphasize adaptive grades rather than unsubstantiated clades.

Euprimates resemble insectivores and microchiropteran bats, but differ from most other placental and all non-placental mammals, in having a promontory artery⁸. This vessel, the homologue of the intracranial part of the human internal carotid artery, characteristically runs in a groove or canal across part of the cochlear promontory. Among Microsyopidae, *Cynodontomys* exhibits a deep promontory sulcus^{9,10} that matches in calibre and position the carotid canals of the earliest euprimates (members of the families Adapidae and Omomyidae). Other plesiadapiform families lack this feature. In known

Plesiadapidae, promontory grooves (Fig. 1) are tiny and highly variable in orientation within and between species^{11,12}. These grooves are more likely to have contained divisions of the tympanic nervous plexus than the promontory artery (which, even if present, would have been vestigial). In known Paromomyidae there are no identifiable promontorial grooves, but there is a longitudinal ridge that runs the length of the middle-ear cavity. In *Phenacolemur* this structure has been tentatively identified as a promontory canal¹³, but as the 'canal' is imperforate in both *Phenacolemur* and the related genus *Ignacius*, the paromomyid forebrain must have been supplied by a vessel other than the promontory artery.

In *Ignacius* the cerebral blood supply appears to have been channelled along the medial side of the auditory region, rather than through it as in a primitive euprimate. This inference is based on the bilateral presence of smooth-walled apertures situated between the sphenoid and bulla in a recently discovered partial cranium of *Ignacius graybullianus* (collected with associated dentition by B. Holly Smith from University of Michigan locality SC-6 in the Clark's Fork Basin, Wyoming; Fig. 2a). These apertures, although distorted *post mortem*, are strikingly similar to the middle lacerate foramina of lorisid and cheirogaleid primates, muroid rodents, dogs and various other placentals^{14–18}. In these groups the middle lacerate foramen transmits arteries that contribute to cerebral circulation: a medially positioned internal carotid, or a branch of the ascending pharyngeal artery, or both^{15,16,19}. The medially positioned internal carotid is thought by some to be a homologue of the promontory artery^{20,21}, but it is more widely regarded as a distinct vessel, the 'medial entocarotid'^{9,13,22}. Szalay²³ considers that both the medial entocarotid and a separate promontory artery were present in early eutherians, but that the first-named vessel was lost in the line leading to primates. Accordingly, he has defined the loss of the medial entocarotid (and its foramen) as a feature marking the boundary between primates and non-primates. If *Ignacius* retained the medial entocarotid, then according to this criterion the genus cannot be primate. If, instead, the medial vessel was an enlarged ascending pharyngeal which supplied the endocranium, *Ignacius*' basicranial vasculature might have been superficially loris-like (Fig. 2b). However, because the arterial arrangement characteristic of modern lorisiforms is almost certainly derived from the more primitive pattern found in early Eocene Adapidae, any special resemblances between lorises and *Ignacius* must represent convergences, of no value in inferring phylogenetic relationships. Neither *Ignacius* nor any other plesiadapiform shares significant vascular homologies with early euprimates.

The medial bullar wall is a lamina continuous with the petrosal bone in all euprimates and adequately known

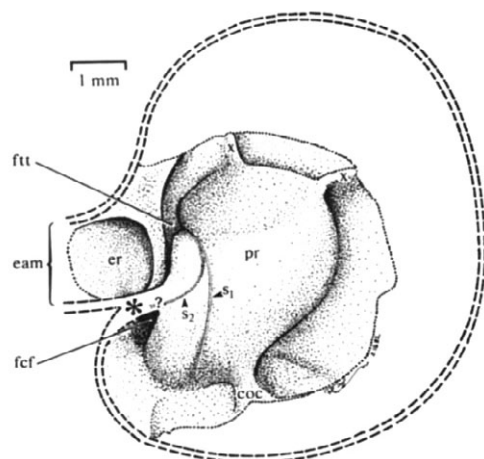


Fig. 1 Variability of promontorial grooves in plesiadapiforms. Semi-schematic ventral view of right petrosal of the plesiadapid *Nannodectes*, based on two incomplete specimens (*N. intermedius*, United States National Museum no. 309902, and *N. gidleyi*, American Museum of Natural History no. 17388). In USNM 309902, there is an apparent vascular sulcus (s_1) originating near the roof of the middle ear cavity; the sulcus does not lead to an external aperture, and we suspect that it carried a nerve (tympanic nerve?) during life. In AMNH 17388 sulcus s_1 does not exist, but there is another (s_2) which is not represented in USNM 309902. Sulcus s_2 grooves, but does not seem to perforate (?), the bridge of bone (*) uniting the promontory and the posterior bullar wall. In specimens of the related genus *Plesiadapis*, the equivalent of s_2 often leads to an aperture which has been identified as the homologue of the posterior internal carotid foramen of lemurs. The variable presence and size of s_2 and its foramen suggests that the internal carotid (even if present) would have been of no functional importance. Middle-ear septa (x), sometimes thought to represent canal-like continuations of these sulci, are imperforate and do not, in any event, connect with the sulci (which seem to end within the fossa for m. tensor tympani). For key to abbreviations, see Fig. 2.

plesiadapiforms (other than microsyopids, in which bulla-petrosal continuity was absent^{9,10}). Among living primates, this continuity reflects the fact that the bulla originates in the fetus as an outgrowth of the ossifying petrosal. This ontogenetic pattern, in its details, distinguishes modern primates from all other extant mammals^{14,24}. However, such patterns can only be properly established by studies of prenatal development. It is not possible to infer anything about bullar composition from an absence of bullar sutures in adults; a number of extant mammals (for example, some rodents²⁵) develop a non-petrosal bulla which fuses seamlessly with the petrosal in postnatal life. Postnatal fusion may or may not have occurred in archaic primates; but while there are no fossils of fetal plesiadapiform ear regions, the question is (and is likely to remain) open.

The available fossil evidence does not support the conclusion that the earliest primates were distinguished from their nearest relatives by a 'tube-enclosed carotid circulation within the petrosal-derived bulla'². The carotid part of this definition excludes known non-microsyopid Plesiadapiformes, and the bulla part is not demonstrable for any fossil primate. It is parsimonious to assume that the ancestral euprimate, like its living descendants, had a petrosal bulla; but the last common ancestor of euprimates and microsyopids did not, and so bullar composition in other archaic primates is not inferable from parsimony considerations. Other attempts²⁶⁻²⁸ to find unique features for the plesiadapiform-euprimate clade have similarly ended in failure^{29,30}, and the position of the lower taxonomic boundary of primates remains controversial³¹.

The search for exclusive features diagnostic for primates might be better replaced by attempts to formulate and account for the significant grade boundaries found within the order.

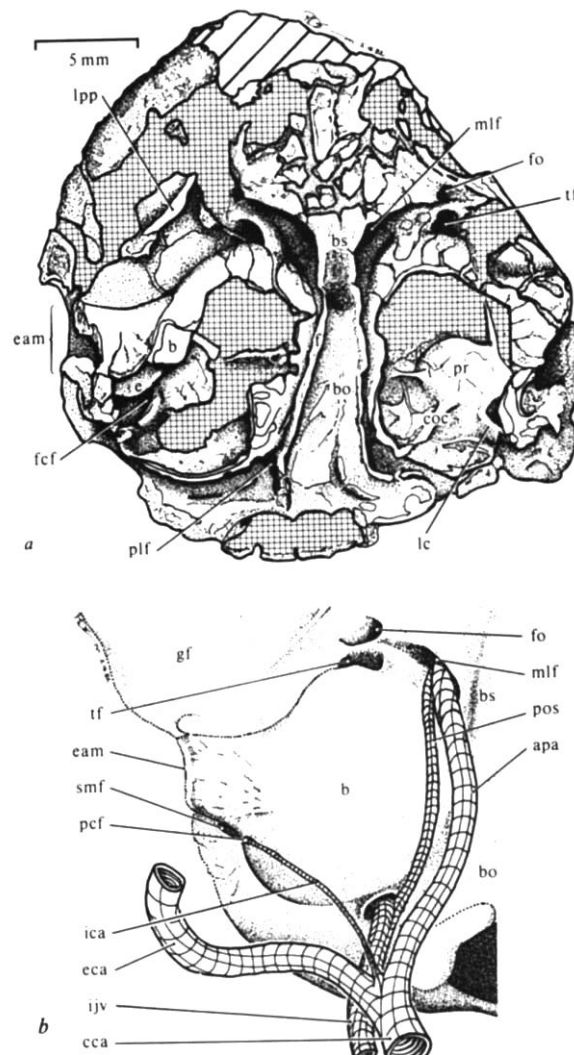
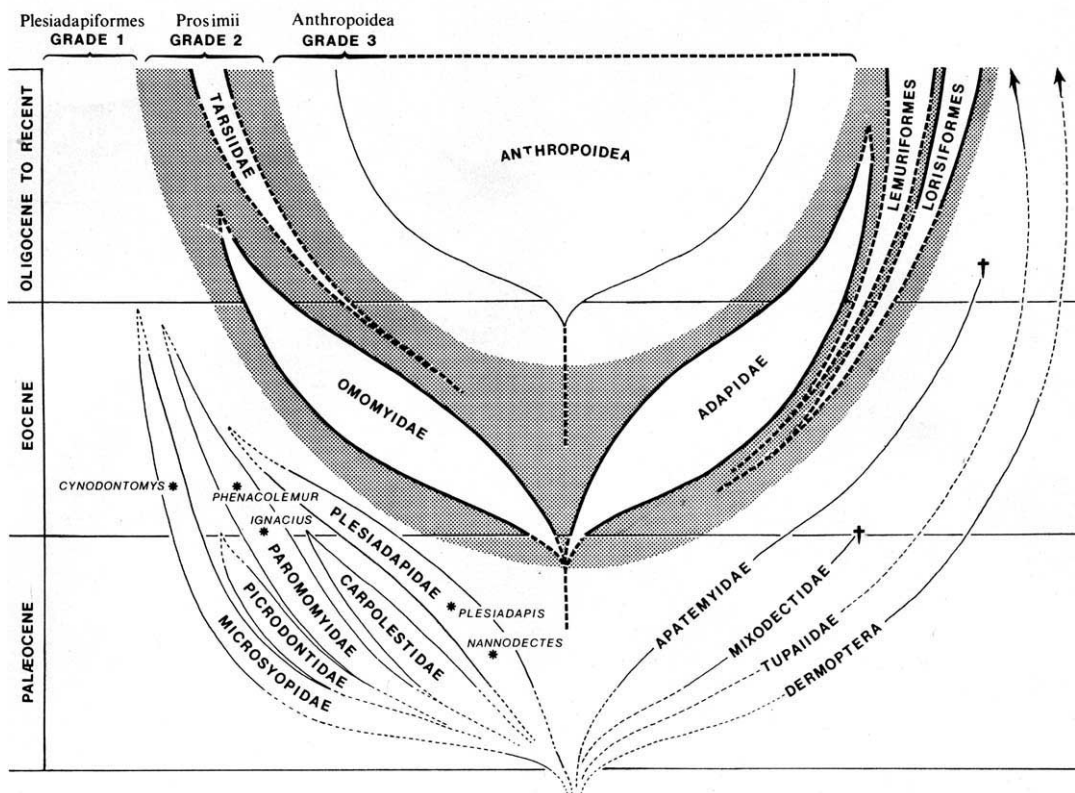


Fig. 2 Ventral view of *Ignacius graybullianus*, University of Michigan Museum of Paleontology no. 68006 (a) and reconstruction of fossil's right auditory region and major vascular channels (b). Key: apa, ascending pharyngeal artery; b, bulla; bo, basioccipital; bs, basisphenoid; cca, common carotid artery; coc, cochlear canalculus; e, medial rim of ectotympanic; eam, external acoustic meatus; eca, external carotid artery; er, epitympanic recess; f, flange on basioccipital; fcf, fossula of the cochlear fenestra; ftt, fossa for tensor tympani muscle; gf, glenoid fossa; ica, internal carotid artery; iju, internal jugular vein; lc, longitudinal ridge; lpp, lateral pterygoid plate; mlf, middle lacerate foramen; pcf, posterior carotid foramen; plf, posterior lacerate foramen; pos, petro-occipital sinus; pr, promontory; smf, stylomastoid foramen; tf, foramen for auditory tube. In a, matrix-filled areas on the crushed and somewhat distorted skull are identified by cross-hatching. In b, vessel reconstruction is based on pattern found in modern lorises and cheirogaleid lemurs; precise calibre of vascular channels and their furcation points are, of course, not known. The fossil does not exhibit a recognizable pcf, and the ica may therefore have been completely absent.

Only two major grade transitions, delimiting the upper and lower boundaries of Prosimii, can be defined at present. The antecedent plesiadapiform grade cannot be precisely defined, and it may even be polyphyletic. Polyphyletic origin of a grade damages its taxonomic status but enhances its evolutionary significance, because a grade boundary crossed by several parallel lineages is correspondingly more likely to reflect adaptation under pervasive selection pressures rather than historical accident. The five plesiadapiform families (Fig. 3, bottom left of diagram) share euprimate-like features of molar morphology that give them particular significance for understanding

Fig. 3 Major primate taxa and possible primate relatives, organized in the form of grades. Temporal distributions of major primate taxa (including plesiadapiform genera mentioned in text) are shown in outline, and unresolved phylogenetic relationships (for example, origin of Anthropeidea, origin of prosimian groups) are purposely left ambiguous. Resemblances of Plesiadapiformes to Omomyidae, formerly regarded by P.D.G. as indications of direct relationship, are now thought to represent convergences³². In cladistic terms, only grade 3 is strictly monophyletic; we acknowledge that grade 2 is paraphyletic, and that grade 1 is polyphyletic (and would probably remain so even if restricted to the families on the as. barriers to analysis of t



primate origins, even if it is not possible to resolve their precise relationships to one another, to euprimates, and to the slightly less 'plesiadapiform' families noted on the right of Fig. 3.

We thank M. C. McKenna, R. J. Emry, P. Houde, D. E. Russell, J.-L. Pellouin, W. Ryan, R. Kay, V. Mahanger-MacPhee, and M. Johnston. Research support was provided by National Science Foundation (grants DEB 82-08797 to M.C. and R.D.E.M.; DEB 80-10846 and BNS 80-16742 to P.D.G.).

Received 20 July; accepted 17 November 1982.

1. Szalay, F. S. in *Phylogeny of the Primates, a Multidisciplinary Approach* (eds Luckett, W. P. & Szalay, F. S.) 91–125 (Plenum, New York, 1975).
2. Szalay, F. S. & Delson, E. *Evolutionary History of the Primates* (Academic, New York, 1979).
3. Szalay, F. S. in *The Functional and Evolutionary Biology of Primates* (ed. Tuttle, R.) 3–35 (Aldine, Chicago, 1972).
4. Hoffstetter, R. *Bull. Mém. Soc. Anthropol. Paris (sér. 13)* **4**, 327–347 (1977).
5. Simpson, G. G. *Am. Mus. Novit.* **816**, 1–30 (1935).
6. Szalay, F. S. *Evolution* **22**, 19–36 (1968).
7. Gingerich, P. D. *Pap. Paleontol. Univ. Michigan Mus. Paleontol.* **15**, 1–140 (1976).
8. Archibald, J. D. *J. hum. Evol.* **6**, 609–622 (1977).
9. McKenna, M. C. *Folia primatol.* **4**, 1–25 (1966).
10. Szalay, F. S. *Bull. Am. Mus. nat. Hist.* **140**, 193–330 (1969).
11. Saban, R. *Mém. Mus. natn. Hist. nat., Paris (sér. A)* **29**, 1–278 (1963).
12. Russell, D. E. *Mém. Mus. natn. Hist. nat., Paris (sér. C)* **13**, 1–324 (1964).
13. Szalay, F. S. *Am. J. phys. Anthropol.* **36**, 59–76 (1972).
14. MacPhee, R. D. E. *Contr. Primatol.* **18**, 1–282 (1981).
15. Cartmill, M. in *Phylogeny of the Primates, a Multidisciplinary Approach* (eds Luckett, W. P. & Szalay, F. S.) 313–354 (Plenum, New York, 1975).
16. Story, H. E. *Fieldiana, Zool.* **32**, 477–557 (1951).
17. Guthrie, D. A. *Bull. Mus. comp. Zool.* **128**, 455–481 (1963).
18. Bugge, J. *Acta anat. (Suppl. 62)* **87**, 1–160 (1974).
19. Butler, H. *Int. J. Primatol.* **1**, 333–343 (1980).
20. Presley, R. *Acta anat.* **103**, 238–244 (1979).
21. Cartmill, M. & MacPhee, R. D. E. in *Comparative Biology and Evolutionary Relationships of Tree Shrews* (ed. Luckett, W. P.) 95–132 (Plenum, New York, 1980).
22. Van Valen, L. *Evolution* **19**, 137–151 (1965).
23. Szalay, F. S. *J. hum. Evol.* **6**, 3–18 (1977).
24. MacPhee, R. D. E. *Folia primatol.* **31**, 23–47 (1979).
25. Klaauw, C. J. van der *Bull. Am. Mus. nat. Hist.* **62**, 1–352 (1931).
26. Szalay, F. S. & Decker, R. L. in *Primate Locomotion* (ed. Jenkins, F. A.) 223–259 (Academic, New York, 1974).
27. Szalay, F. S., Tattersall, I. & Decker, R. L. *Contr. Primatol.* **5**, 136–166 (1975).
28. Szalay, F. S. & Drawhorn, G. in *Comparative Biology and Evolutionary Relationships of Tree Shrews* (ed. Luckett, W. P.) 133–169 (Plenum, New York, 1980).
29. Lewis, O. J. *J. Anat.* **131**, 275–298 (1968).
30. Novacek, M. J. in *Comparative Biology and Evolutionary Relationships of Tree Shrews* (ed. Luckett, W. P.) 35–93 (Plenum, New York, 1980).
31. Cartmill, M. in *A History of American Physical Anthropology 1930–1980* (ed. Spencer, F.) 147–186 (Academic, New York, 1982).
32. Gingerich, P. D. *J. hum. Evol.* **10**, 345–374 (1981).

Chemolithoautotrophic metabolism of anaerobic extremely thermophilic archaeobacteria

F. Fischer*, W. Zillig†, K. O. Stetter* & G. Schreiber†

* Universität Regensburg, Lehrstuhl für Mikrobiologie,
8400 Regensburg, FRG

† Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG

Several types of extremely thermophilic archaeobacteria have recently been isolated from solfataric water holes, hot springs and hot sea floors¹⁻⁶. It has been shown that some of them can live using sulphur respiration of reduced carbon substrates as a source of energy, a type of metabolism previously described for the eubacterium *Desulfuromonas*⁷. We report here that several extremely thermophilic archaeobacteria can live with carbon dioxide as their sole carbon source, obtaining energy from the oxidation of hydrogen by sulphur, producing hydrogen sulphide. They are thus capable of a new type of anaerobic, purely chemolithoautotrophic metabolism, a possible primaeval mode of life.

The conservation of energy from the oxidation of molecular hydrogen with elemental sulphur by *Spirillum* 5175 (ref. 8), and the formation of H₂S from hydrogen and sulphur by suspensions of *Campylobacter* sp. (ref. 9) are evidence that H₂S formation can be the basis of a chemolithotrophic existence, though acetate is required as a carbon source in the first instance, and no coupling to growth has been demonstrated in the latter.

The archaeobacterium *Thermoproteus tenax* Kral (DSM 2078) is able to grow with carbon monoxide as sole carbon source, provided that elemental sulphur is available as terminal electron acceptor^{1,10}. Equivalent amounts of H₂S and CO₂ are produced.

Many isolates of *Thermoproteus* from Icelandic and North American solfataras, among them *T. tenax* and *Thermoproteus neutrophilus* (F. F., K.O.S. and W.Z., unpublished), and the yet unnamed isolate H3 (Fig. 1a), can even grow in the presence

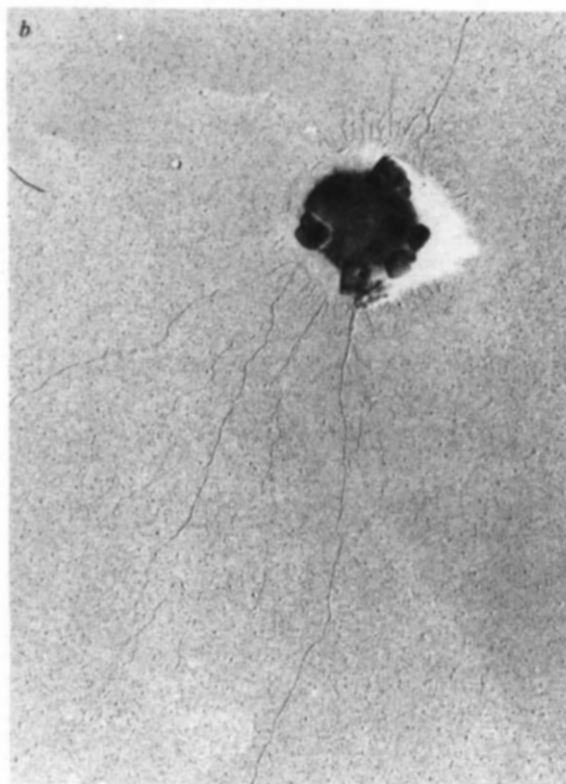
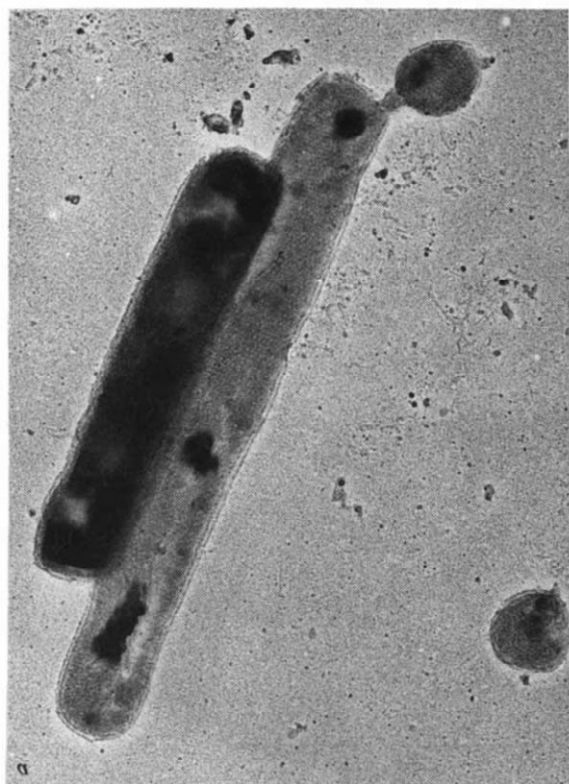
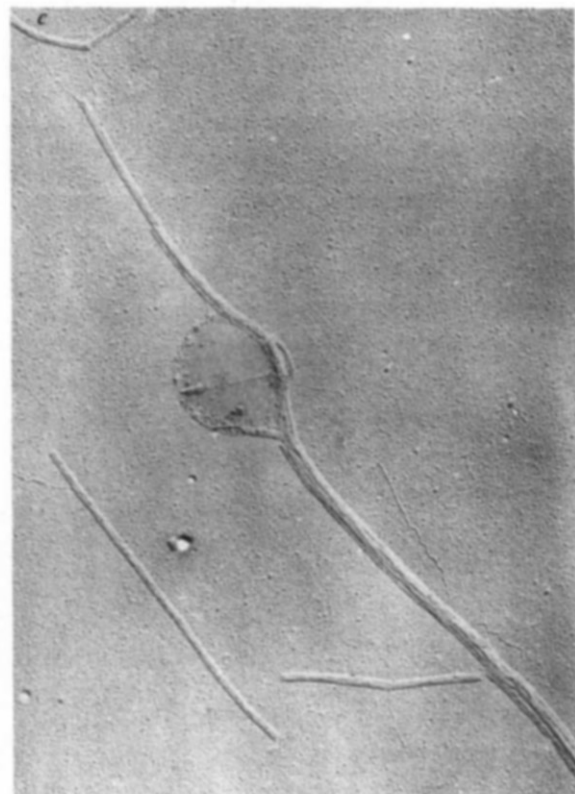


Fig. 1 Electron micrographs of shadowed specimens of: *a*, *Thermoproteus* sp. H3, 'golf club' form, and released daughter cell; *b*, *Thermodiscus maritimus*; *c*, *Pyrodictium occultum*. Scale bar, 1 μ m.



of molecular hydrogen and sulphur, with CO_2 as sole carbon source. Sulphur is consumed; no methane, but approximately 1.5 moles H_2S per g cell mass (dry weight)—at approximately 10^9 cells per ml culture—were produced in these conditions. At a temperature of 88°C , and when the culture was agitated and gassed with a mixture of 80% H_2 and 20% CO_2 , the generation time was 4–8 h. A progressive uncoupling of growth and H_2S production was observed during approach to the stationary state. Whereas *T. tenax* and the isolate H3 are facultative chemolithoautotrophs, alternatively able to grow by the sulphur respiration of organic matter, *T. neutrophilus* is an obligate autotroph (see Table 1).

Two yet unnamed, different types of organisms isolated from extremely hot submarine solfataric springs and growing in sea water in the presence of sulphur, H_2 and CO_2 , show the same obligate chemolithoautotrophy. *Thermodiscus maritimus* is a moderately halophilic extremely flat disk with a thick envelope and long protrusions (Fig. 1*b*)⁵; *Pyrodictium occultum* forms a fragile network of thin hyphae with attached disks (Fig. 1*c*), growing optimally at 105°C ⁶.

The utilization of sulphur by all these organisms is accompanied by a change of the state of the sulphur apparent by a change of its colour from light yellow to greyish green, and by a very fine and even distribution in the medium. All these organisms are archaeobacteria¹¹. *Thermoproteus* belongs to the recently defined novel order Thermoproteales, which, together with the order represented by *Sulfolobus*, forms a distinct division of the urkingdom of the archaeobacteria previously termed the thermoacidophilic branch^{8,12,13}. As some of its members tolerate or even prefer pH values near neutrality we propose to rename it the sulphur metabolizing branch. 16S ribosomal RNA sequences (E. Stackebrandt and K.O.S., unpublished) and 16S rRNA–DNA cross-hybridization data place *Thermodiscus* and *Pyrodictium* in the same branch, though their exact taxonomic positions remain to be determined.

The solfataric biotopes, of *Thermoproteus* as well as of the marine sulphur metabolizing archaeobacteria, are hot, rich in

sulphur, and usually strongly gassed by superheated steam known to contain CO_2 , H_2 and possibly carbon monoxide¹⁴. Such conditions may well have existed early in Earth's history. Hence, this type of chemolithoautotrophy, the related sulphur respiration, and the mechanisms involved could be primaevial, and should be considered possible precursors of other types of hydrogen oxidation or respiration.

Within the solfataric habitat, the widely distributed *Thermoproteus* is another important primary producer of organic matter in addition to the aerobic *Sulfolobus*, which oxidizes H_2S

Table 1 Properties of thermophilic, chemolithoautotrophic, sulphur metabolizing archaeobacteria

Organism	Source	Source temperature (°C)	Source pH	Optimal growth temperature (°C)	Optimal growth pH	Autotrophy
<i>Thermoproteus tenax</i> Kra 1, DSM 2078	Krafla, Iceland, mud hole	93	6.0	88	5, 5	Facultative
<i>Thermoproteus neutrophilus</i> Hv 24 (V24), DSM 2338	Kerlingarfjoll, Iceland, hot spring	85	6.5	85	6, 8	Obligate
<i>Thermoproteus</i> sp. H3	Hengill, Iceland, mud from 0.5 m depth	98	5.5	90	5, 5	Facultative
<i>Thermoproteus</i> sp. Bu5	Mt Lassen, California, USA, hot spring	90	4.5	88	5, 2	Facultative
<i>Thermotoga maritima</i> S2	Vulcano, Italy, hot sea water	85	6.5	85	6, 5	Obligate
<i>Pyrodicticum occultum</i> PL 19	Vulcano, Italy, hot sea floor	103	6.5	105	6, 5	Obligate

Thermoproteus species were grown under strong agitation in Allen's medium¹⁷ containing 5 g l⁻¹ sulphur, and gassed with 80% H₂ and 20% CO₂ at pH 5.5, or, in the case of *T. neutrophilus*, pH 6.5. The marine isolates were grown without agitation in sea water, with 5 g l⁻¹ sulphur and gassed with 80% H₂ and 20% CO₂.

to sulphur, and finally to sulphuric acid¹⁵, and thermophilic methanogens¹⁶. These chemolithoautotrophs provide a solid basis for the occurrence and remarkable frequency of several heterotrophic organisms, for example *Thermophilum*² and *Desulfurococcus*³ in this niche.

Received 4 November; accepted 2 December 1982.

1. Zillig, W. et al. *Zbl. Bakt. Hyg., I. Abt. Orig.* **C2**, 205–227 (1981).
2. Zillig, W. et al. *System. appl. Microbiol.* formerly *Zbl. Bakt. Hyg., I. Abt.* **4**, 79–87 (1983).
3. Zillig, W. et al. *Zbl. Bakt. Hyg., I. Abt. Orig.* **C3**, 304–317 (1982).
4. Zillig, W., Holz, I., Janeković, D., Schäfer, W. & Reiter, W. D. *System. appl. Microbiol.* formerly *Zbl. Bakt. Hyg., I. Abt.* **4**, 88–94 (1983).
5. Stetter, K. O., König, H. & Stackebrandt, E. *System. appl. Microbiol.* formerly *Zbl. Bakt. Hyg., I. Abt. Orig.* **4** (in the press).
6. Stetter, K. O. *Nature* **300**, 258–260 (1982).
7. Pfennig, N. K., Biehl, H. *Archs Microbiol.* **110**, 3–12 (1976).

We thank the Deutsche Forschungsgemeinschaft for generous support, H. König, E. Klemaier and D. Janeković for the electron micrographs, A. Rechenmacher and I. Holz for technical assistance, and the Icelandic Research Council for a research permit.

8. Wolfe, R. S. & Pfennig, N. *Appl. env. Microbiol.* **33**, 427–433 (1977).
9. Laanbroek, H. J., Stal, L. J. & Veldkamp, H. *Archs Microbiol.* **119**, 95–102 (1978).
10. Zillig, W., Schnabel, R., Tu, J. & Stetter, K. O. *Naturwiss.* **69**, 197–204 (1982).
11. Fox, G. E. et al. *Science* **209**, 457–463 (1980).
12. Tu, J. et al. *J. molec. Evol.* **18**, 109–114 (1982).
13. Zillig, W., Tu, J. & Holz, I. *Nature* **293**, 85–86 (1981).
14. Williams, H. & McBirney, A. R. in *Vulcanology* 319–346 (Freeman, San Francisco, 1979).
15. Brock, T. D., Brock, K. M., Belley, R. T. & Weiss, R. L. *Archs Microbiol.* **84**, 54–68 (1972).
16. Stetter, K. O. et al. *Zbl. Bakt. Hyg., I. Abt. Orig.* **C2**, 166–178 (1981).
17. Allen, M. B. *Archs Microbiol.* **32**, 270–277 (1959).

Interocular transfer in cats with early callosal transection

Maurice Ptito

Groupe de Recherche en Neuropsychologie Expérimentale, Université du Québec à Trois-Rivières, CP 500, Trois-Rivières, Québec G9A 5H7, Canada

Franco Lepore

Département de Psychologie et Centre de Recherche en Sciences Neurologiques Université de Montréal, CP 6128, Montréal, Québec H3C 5J7, Canada

The visual system is capable of reorganization following experimental interventions, provided that these are carried out before the 'critical period' early in life^{1–3}. Direct evidence suggests that this plasticity may also apply to the commissural system^{4–8}. In the present experiment, we sectioned the posterior two-thirds of the corpus callosum in kittens either before this structure attained maturity, as defined by various anatomical and physiological parameters, or after callosal maturation but before the end of the critical period for most other visual functions. As adult, further transection of the optic chiasma was carried out and these animals were then compared with adult split-brain cats for their ability to transfer monocularly learned pattern discriminations from one hemisphere to the other. Our results indicate that only the first group of animals with the earlier callosal transection demonstrated significant interhemispheric transfer. This suggests that the secondary commissural system is subject to at least some degree of functional plasticity. However, its critical period is very brief and possibly pre-dates the optimal activation of the callosal pathway.

The three groups used in the experiment each contained two animals. The corpus callosum is both anatomically and physio-

logically immature at birth and only attains adult-like characteristics after 4 or 5 weeks of life^{9–12}. The callosum was therefore sectioned in the first group of cats when they were 20 days old, that is, before callosal maturation. In the second group it was sectioned at 45 days because although corpus callosum is mature, experimental manipulations can still produce structural and/or functional modifications in many parts of the visual system when carried out before the end of the eighth or ninth week of life^{1–3}. The animals were raised in the colony and when they became mature adults (8–9 months of age) their optic chiasma was sectioned so that each eye was connected to only one hemisphere. A similar split-brain preparation was carried out on two normal adults from the colony. In all cases histological studies confirmed the effectiveness of the callosal and chiasma transections (Fig. 1).

After appropriate pretraining, the six cats learned monocularly in a Thompson-like two-choice apparatus to discriminate between two patterns which differed either in orientation or in configuration¹³. An animal had to choose one pattern (the positive stimulus) and avoid a second pattern (the negative stimulus). It indicated its preference by pushing on the pattern with its nose. This caused the door on which the pattern was affixed to open, giving the animal access to the reward. On achieving a rather strict learning criterion (36 correct responses in two consecutive sessions of 40 trials each), the eye patch was switched and the animal then learned the discrimination with the other eye. Any savings with this eye implied an interhemispheric transfer of training. Once this criterion was achieved with this second eye, a new pair of stimuli was chosen and the procedure repeated. In all, four pairs of discriminations were learned by each cat, two using the left eye for learning and the right eye for transfer testing and two using the inverse procedure.

Representative learning curves for each cat and for one pair of patterns are presented in Fig. 2. Individual data obtained

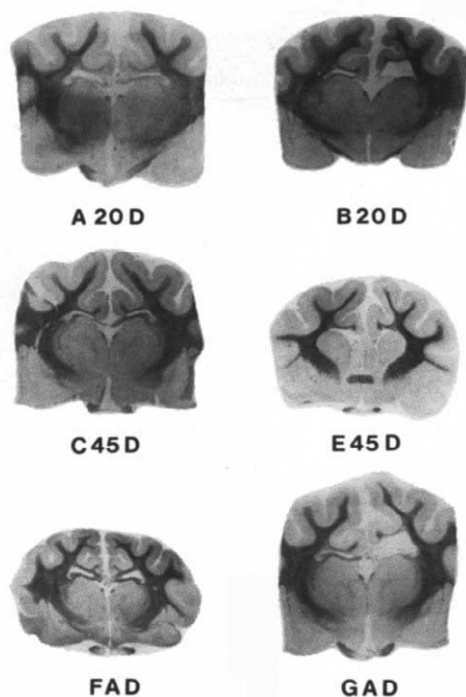


Fig. 1 Coronal sections through the brain of the six cats showing the completeness of the callosal and chiasmatic transections. The callosotomy was performed for the top two sections at 20 days, for the middle two sections at 45 days, and for the bottom two sections at adulthood.

from the two cats in each group on the four pairs of pattern discriminations were pooled for further analyses. To evaluate the degree of interocular transfer, two measures, based on the criteria generally used in this type of study, were used. The first compared the performance of the animal during the first session when each eye was tested on a pattern for the first time. The eye used for learning should show chance performance, namely, 20 errors in 40 trials. Perfect transfer would be demonstrated by an animal immediately performing above the criterion level when the pattern is shown for the first time to the second eye. Chance performance would indicate that no transfer had taken place. However, although no transfer may be detected, it is still possible that the untrained side could have benefited from the experience given to the first eye. To evaluate this the number of errors in relation to the criterion level using the untrained eye was determined.

The results obtained using these two methods of analysis are presented in Fig. 3. Various conclusions can be drawn from these results. First, the average number of trials needed to learn to discriminate between the four pairs of patterns is similar for the three groups. This shows that the early callosal sections did not affect the ability of the animals to learn this type of discrimination compared with adult split-brain preparations. Second, the results show that, as could be expected from previous studies¹⁴⁻¹⁷, there was no transfer in the adult split-brain animals. Interestingly, animals callosotomized at 45 days behaved in a similar manner. The 20-day callosal sectioned cats, on the other hand, showed an average of about 58% fewer errors when learning the discriminations with the second eye than with the first.

Our results show that the commissural system, like many other visual structures, is capable of functional plasticity. However, this period of susceptibility is very brief and seems to end, at least for the tasks required in this experiment, once the larger corpus callosum becomes anatomically and physiologically mature. The pathways through which this recovery becomes possible during the critical period are unknown and we are presently studying this problem. The two most likely are: (1) the intercollicular commissure, which normally joins

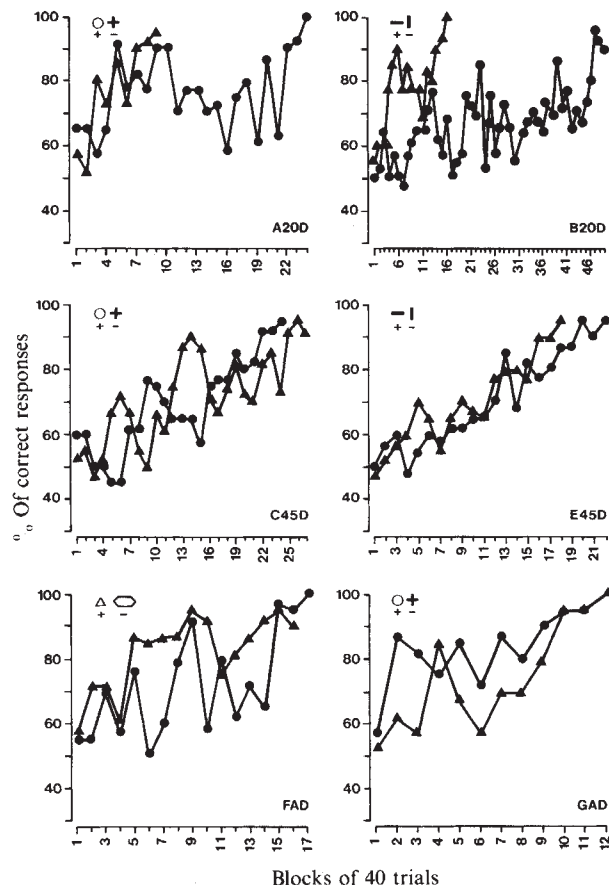


Fig. 2 Representative learning (●) and transfer (▲) curves for each subject in each group for one of the four pattern discriminations. The abscissa indicates the number of blocks of 40 trials and the ordinate give the % correct responses for the session. The pattern used is shown in the upper left corner of each pair of curves.

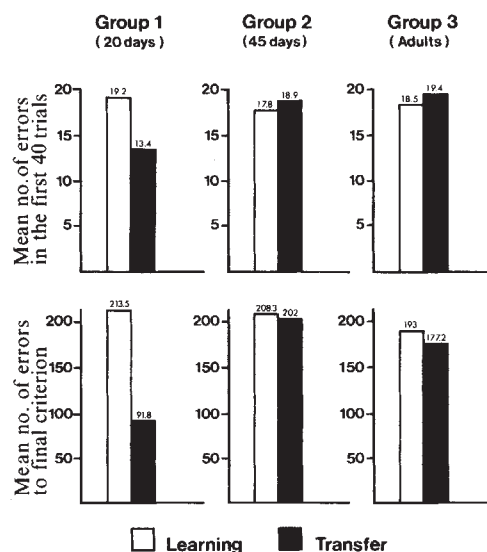


Fig. 3 Bar charts summarizing learning (light bars) and transfer (dark bars) scores obtained for each group following section of the corpus callosum and the optic chiasma. Each histogram represents the mean number of errors in the first 40 trials (upper series) and the mean number of errors to final criterion (lower series).

two visual structures¹⁸ and which may, in the absence of the callosum, become functionally more effective¹⁹ and (2) the anterior commissure, which transmits visual information across the hemispheres in monkeys^{20,21} and man^{22,23}. Although the anterior commissure does not appear to join visual structures in the cat or participate in interhemispheric transfer of visual information in the adult split-brain animal, the appearance of such a function in primates and the rather limited data from strychnine neuronography²⁴ showing a projection through it

from the posterior suprasylvian area, suggest it should be examined in further studies of alternative pathways mediating interhemispheric transfer in early callosotomized cats. The results obtained in this experiment perhaps explain the lack of disconnection syndrome found in human callosal agenesis^{25,26} and in callosotomized children²⁷.

This research was supported by grants from le Ministère de l'Éducation du Québec (FCAC) and le Conseil de la Recherche en Sciences Naturelles et en Génie du Canada (CRSNG).

Received 17 August; accepted 30 November 1982.

- Hubel, D. H. & Wiesel, T. V. *J. Neurophysiol.* **30**, 1561–1573 (1970).
- Blakemore, C. & van Sluyters, R. C. *J. Physiol., Lond.* **237**, 195–216 (1974).
- Cynader, M. & Mitchell, D. E. *Nature* **270**, 177–178 (1977).
- Cynader, M., Lepore, F. & Guillemot, J. P. *Nature* **290**, 139–140 (1981).
- Cynader, M., Lepore, F., Guillemot, J. P. & Fera, M. *Rev. Can. Biol.* **40**, 51–59 (1981).
- Lund, R. D., Mitchell, D. E. & Henry, G. H. *Brain Res.* **149**, 169–172 (1978).
- Innocenti, G. M. & Frost, D. O. *Nature* **280**, 231–234 (1979).
- Schatz, C. J. *J. comp. Neurol.* **173**, 497–518 (1977).
- Lund, R. C. & Mitchell, D. E. *Brain Res.* **167**, 172–175 (1979).
- Grafstein, B. J. *Neurophysiol.* **36**, 79–99 (1963).
- Anker, R. L. & Cragg, B. G. *J. comp. Neurol.* **154**, 29–42 (1974).
- Innocenti, G. M. *Arch. ital. Biol.* **116**, 463–470 (1978).
- Pitro, M., Lepore, F., Lassonde, M., Miceli, D. & Guillemot, J.-P. *Rev. Can. Biol.* **40**, 67–74 (1981).

- Myers, R. E. *Brain* **79**, 358–363 (1956).
- Myers, R. E. & Sperry, R. W. *Anat. Rec.* **115**, 351–352 (1953).
- Sperry, R. W., Stamm, J. G. & Miner, N. M. *J. comp. Physiol. Psychol.* **49**, 529–533 (1956).
- Voneida, T. J. *Expl Neurol.* **8**, 493–504 (1963).
- Sprague, J. M., Berlucchi, G. & Diberardino, A. *Brain Behav. Evol.* **3**, 285–294.
- Luybimov, N. N. *Fedn Proc.* **24**, T1011–T1014 (1965).
- Black, P. & Myers, R. E. *Science* **146**, 799–800 (1964).
- Doty, R. W. *Physiol.* **9**, 170 (1966).
- Sperry, R. W. *Am. Psychologist* **23**, 723–733 (1968).
- Risse, G. L., Ledoux, J., Springer, S. P., Wilson, D. H. & Gazzaniga, M. S. *Neuropsychologia* **16**, 23–31 (1978).
- Garol, H. W. *J. Neurophysiol.* **1**, 422–429 (1942).
- Saul, R. E. & Sperry, R. W. *Neurology* **18**, 307 (1968).
- Sauerwein, H. & Lassonde, M. *Neuropsychologia* (in the press).
- Geoffroy, G., Lassonde, M., Sauerwein, H. & Flessas, J. *Soc. Neurosci. Abstr.* **7**, 382 (1981).

Nerve growth factor antagonizes the neurotoxic action of capsaicin on primary sensory neurones

Uwe Otten*, H. P. Lorez† & F. Businger*

* Department of Pharmacology, Biocentre of the University, CH-4056 Basel, Switzerland

† Pharmaceutical Research Department, F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel, Switzerland

The protein nerve growth factor (NGF) is a naturally occurring trophic substance for sympathetic neurones^{1–3} and for at least those primary sensory neurones containing substance P (refs 4–6). Thus retrogradely transported NGF increased substance P and protein content in corresponding dorsal root ganglia⁶. Moreover, anti-NGF antibodies administered to newborn rats decreased substance P and somatostatin levels in dorsal root ganglia and dorsal spinal cord^{4,5,7}, suggesting an important role for NGF in the postnatal development of peptidergic sensory neurones. These neurones appear to be selectively affected by the neurotoxin capsaicin (8-methyl-N-vanillyl-6-nonenamide)⁸. Treatment of newborn rats with capsaicin led to degeneration of primary sensory neurones containing substance P, somatostatin, vasoactive intestinal polypeptide and cholecystokinin^{9–16}. The mechanism by which capsaicin evokes its neurotoxic effect is unknown. We report here that in newborn rats concomitant administration of NGF partially antagonized the deleterious effect of capsaicin on substance P-containing neurones in dorsal root ganglia as assessed by morphological and biochemical criteria. We conclude that capsaicin destroys the perikarya of primary sensory peptidergic neurones by interfering with the action of NGF, probably by blocking its retrograde axonal transport.

NGF (2.5 S) was prepared from submaxillary glands of adult male mice according to the method of Bocchini and Angeletti¹⁷. Capsaicin (Sigma) was dissolved in 10% ethanol–10% Tween-80 (v/v) in 0.9% (w/v) saline. Pregnant Sprague-Dawley rats were housed singly in transparent plastic cages on a 12-h light/dark cycle, at a constant temperature of 23 °C and with free access to food pellets and tap water. As soon as delivered, each litter was reduced to 10 pups. All injections (subcutaneously, s.c.) were started on the second day of life as follows: one group was injected with a single dose of 40 µg g⁻¹ capsaicin, a second group with 0.5 µg per g NGF (in saline) which was repeated daily for a further 9 days, a third group with a single dose of capsaicin (40 µg g⁻¹) together with NGF

(0.5 µg g⁻¹) daily for 10 days, and a fourth group with the vehicle solutions. All morphological and biochemical experiments were carried out on day 14.

Table 1 shows the number of dorsal root ganglion neurones at various segmental levels after neonatal treatment with NGF, capsaicin or NGF and capsaicin. Treatment with capsaicin reduced the number of neuronal cell bodies in lumbar dorsal root ganglia by almost 50% due to the disappearance of the small to medium-sized dark (B-type) neurones. This effect was partially, but significantly, antagonized by treatment with NGF. NGF treatment alone did not significantly change the neuronal number as found by Kornblum *et al.*¹⁸ (Table 1).

The appearance of substance P staining is shown in Fig. 1. Ganglia from control animals contained many substance P-immunoreactive cell bodies and beaded fibres showing weak to moderate staining intensity (Fig. 1a). Immunoreactive structures were very rare after treatment with capsaicin, but the staining intensity remained unchanged (Fig. 1b, Table 2). After treatment with NGF, staining intensity was moderate to strong as compared with controls, but the number of stained cell bodies per profile unit area appeared almost unchanged (Fig. 1c, Table 2). The staining intensity of substance P cells and fibres was also increased, though to a lesser degree, after combined treatment with capsaicin and NGF; the number of substance P cell bodies per unit area was decreased as compared with controls but significantly increased as compared with capsaicin treatment alone (Fig. 1d, Table 2).

The immunohistochemical data were confirmed and extended by biochemical studies (Fig. 2). Treatment with NGF led to an increase and treatment with capsaicin to a decrease in ganglionic substance P content similar to that reported previously^{4,10–12,19}. The marked decrease (65%) of substance P content in dorsal root ganglia induced by capsaicin was significantly antagonized by concomitant application of NGF. This effect of NGF seems to be permanent, because after combined treatment with capsaicin and NGF, ganglionic levels of substance P were not significantly reduced over the following 3 weeks. Recent studies have shown that capsaicin significantly depleted somatostatin, vasoactive intestinal polypeptide and cholecystokinin as well as substance P in rat dorsal root ganglia and dorsal spinal cord, indicating that the neurotoxic action of capsaicin is not specific for substance P-containing primary sensory neurones^{9–16}. Similarly, NGF partially antagonized the effect of capsaicin on both substance P- and somatostatin-containing neurones (U.O. and H.P.L., unpublished). It is not clear whether the antagonistic action of NGF to capsaicin was restricted to substance P- (and somatostatin-) containing sensory neurones or was common to other peptidergic sensory neurones. The latter possibility is supported by our recent observation that NGF increased

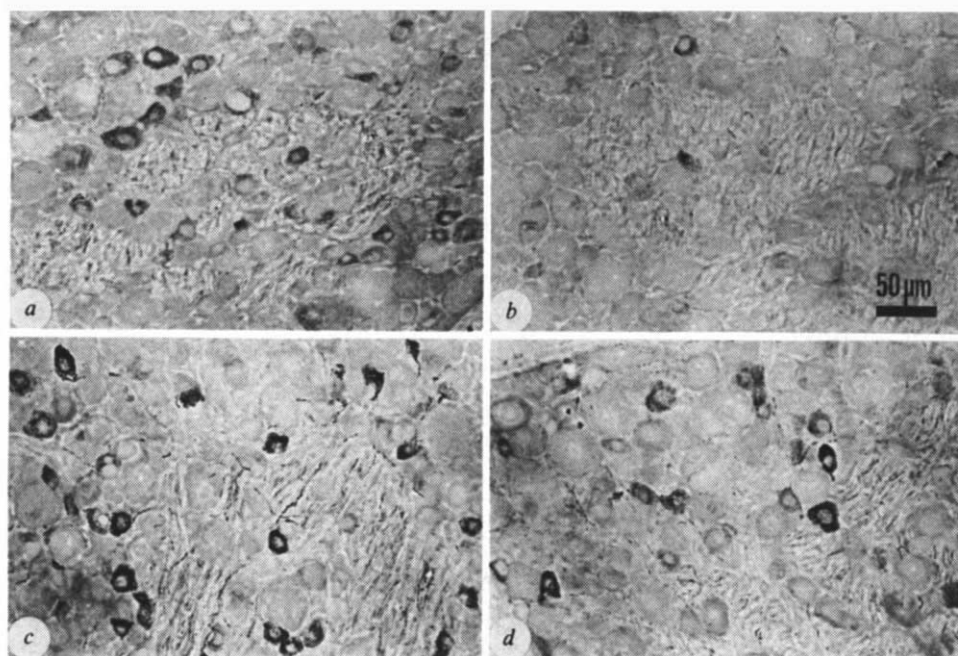


Fig. 1 Substance P immunoreactivity in dorsal root ganglia of rats treated neonatally with vehicle (a), capsaicin (b), NGF (c), or capsaicin and NGF (d). Two-day old rats were injected s.c. with 20 μ l of either vehicle, capsaicin (40 μ g g^{-1}), NGF (0.5 μ g g^{-1}) or capsaicin and NGF. Capsaicin was injected once, NGF daily for 10 days. On day 14 lumbar dorsal root ganglia were examined for substance P immunoreactivity as described in the text to Fig. 2.

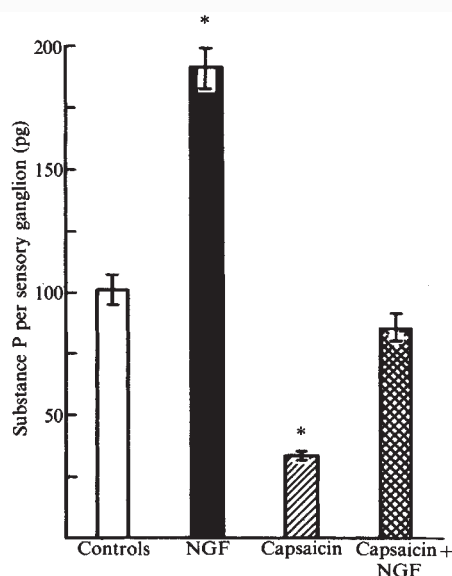


Fig. 2 Effects of NGF, capsaicin or NGF and capsaicin on substance P levels in rat dorsal root ganglia. Two-day-old rats were injected s.c. with 20 μ l of either vehicle, NGF (0.5 μ g g^{-1}), capsaicin (40 μ g g^{-1}) or NGF and capsaicin. On day 14 lumbar dorsal root ganglia were examined for substance P content. Each value represents the mean $\pm$ s.e.m. ($n = 8-10$ animals). For the measurement of substance P levels animals were decapitated and the spinal ganglia (Th12-L5) removed. Substance P immunoreactivity was extracted in 2.0 M acetic acid for 2×30 s in a microwave oven, followed by ultrasonication and centrifugation at 5,000g for 15 min. The supernatant was lyophilized and resuspended in assay buffer for determination by specific radioimmunoassay as previously described by Mroz *et al.*²⁷ using [125 I-Tyr⁸] substance P as tracer. The lower limit of sensitivity of the radioimmunoassay was 2 fmol. * Differs from control at $P < 0.005$.

Table 1 Number of dorsal root ganglion neurones at segmental levels (right L3-L5)

Treatment					L3-L5
	L3	L4	L5	Sum of each rat	$\bar{X} \pm$ s.e.m. of all ganglia
a Control	4,623 $\pm$ 115	6,241 $\pm$ 151	6,528 $\pm$ 200	17,392 $\pm$ 219	5,797 $\pm$ 307 100%
b NGF	4,795 $\pm$ 53	6,379 $\pm$ 259	7,030 $\pm$ 263	18,204 $\pm$ 51	6,068 $\pm$ 349 105%
c Capsaicin	2,132 $\pm$ 141	3,484 $\pm$ 296	4,075 $\pm$ 436	9,691 $\pm$ 136	3,230 $\pm$ 328 56%
d NGF + Capsaicin	3,723 $\pm$ 253	4,800 $\pm$ 211	5,320 $\pm$ 400	13,843 $\pm$ 258	4,614 $\pm$ 279 80%

Values given represent the mean $\pm$ s.e.m. of three animals. For the statistical analysis by Mann-Whitney test, all nine single values per group obtained from three animals with three ganglia (right L3-L5) each, were used (see last column; $2P > 0.05$ for $a : b$, < 0.01 for $d : a$, $d : b$ and $d : c$, < 0.001 for $c : a$ and $c : b$). Two-day-old rats were injected s.c. with 20 μ l of vehicle, NGF (0.5 μ g $d : s$), capsaicin (40 μ g per g^{-1}) or NGF and capsaicin. Capsaicin was injected once, NGF daily for 10 days. On day 14 neuronal cell numbers were determined. For neuronal counts the right dorsal root ganglia (L3-L5) from three rats per group were removed, fixed in Susa solution and embedded in paraffin. Serial sections were cut at 10 μ m and stained with toluidine blue. In every eighth section all neurones containing nucleoli were counted at a magnification of $\times 250$ using the ocular square method. The total number was obtained by multiplying the summed samples by eight, and corrected for split nucleoli using formula four in Königsmark²⁴; multiple nucleoli were accounted for by dividing the correction by the mean number of nucleoli in samples of 70 nuclei. Final correction factors ranged from 0.59 to 0.65.

the content of immunoreactive vasoactive intestinal polypeptide and cholecystokinin in the cell bodies of primary sensory neurones²⁰.

The importance of endogenous NGF or an NGF-like molecule for the development of both substance P and somatostatin sensory neurones is clearly shown by the effects of anti-NGF antibodies^{4,5,7,21,22}. Administration of anti-NGF antibodies to newborn rats results in a marked, but temporary reduction of substance P and somatostatin contents in dorsal root ganglia and spinal cord^{4,5,22}. Prenatal exposure to anti-NGF antibodies produces a drastic reduction in total protein content of dorsal root ganglia and in their peptide content⁵. Using fluorescent rhodamine-conjugated NGF, Levi *et al.*²³ have shown that NGF binds specifically to the plasma membrane surface at sensory nerve terminals, is internalized, retrogradely transported and accumulates in the cell body where it probably regulates peptide content. Although the site and mechanism of action of capsaicin are unknown, the present data indicate that capsaicin destroys the perikarya of peptidergic primary sensory neurones by depriving it of NGF. This can be prevented by exogenous administration of NGF acting directly on the cell body. This assumption is supported by our results showing that axonally applied capsaicin significantly inhibits the retrograde axonal

transport of ¹²⁵I-labelled NGF in primary sensory neurones (U.O., unpublished results). However, it cannot be excluded that capsaicin interferes in another way with the trophic action of NGF on sensory neurones, or that processes triggered by exogenous NGF antagonize and/or compensate directly effects of capsaicin leading to cell death. Perhaps the latter possibility is favoured by the fact that capsaicin degenerates primary sensory neurones within a very short period of time⁸.

This work was supported by the Swiss National Foundation for Scientific Research (grant 3.077-081).

Received 25 October; accepted 26 November 1982.

1. Thoenen, H. & Barde, Y. A. *Physiol. Rev.* **60**, 1284-1335 (1980).
2. Hendry, J. A., Stoeckel, K., Thoenen, H. & Iversen, L. L. *Brain Res.* **68**, 103-121 (1974).
3. Paravicini, U., Stoeckel, K. & Thoenen, H. *Brain Res.* **84**, 279-291 (1975).
4. Otten, U., Goedert, M., Mayer, N. & Lembeck, F. *Nature* **287**, 158-159 (1980).
5. Otten, U., Peck, M., Businger, F., Schlumpf, M. & Lichtensteiger, W. in *Monographs in Neural Sciences* (Karger, Basel, in the press).
6. Goedert, M., Stoeckel, K. & Otten, U. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5895-5898 (1981).
7. Otten, U. *Naunyn-Schmiedeberg's Archs Pharmacol. Suppl.* **319**, 74 (1982).
8. Jancsó, G., Király, E. & Jancsó-Gábor, A. *Nature* **270**, 741-743 (1977).
9. Kessler, J. A. & Black, I. B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4644-4647 (1981).
10. Jessell, T. M., Iversen, L. L. & Cuello, A. C. *Brain Res.* **152**, 183-188 (1978).
11. Nagy, J. I. *et al. Brain Res.* **186**, 435-444 (1980).
12. Gamse, R., Holzer, P. & Lembeck, F. *Br. J. Pharmacol.* **68**, 207-213 (1980).
13. Gamse, R., Leeman, S. E., Holzer, P. & Lembeck, F. *Naunyn-Schmiedeberg's Archs Pharmacol.* **317**, 140-148 (1981).
14. Cuello, A. C., Gamse, R., Holzer, P. & Lembeck, F. *Naunyn-Schmiedeberg's Archs Pharmacol.* **315**, 185-194 (1981).
15. Nagy, J. I., Hunt, S. P., Iversen, L. L. & Emsen, P. C. *Neuroscience* **6**, 1923-1934 (1981).
16. Jancsó, G. *et al. J. Neurocytol.* **10**, 963-980 (1981).
17. Bocchini, V. & Angeletti, P. U. *Proc. natn. Acad. Sci. U.S.A.* **64**, 787-794 (1969).
18. Kornblum, H. I. & Johnson, E. M. *Brain Res.* **234**, 41-51 (1982).
19. Kessler, J. A. & Black, I. B. *Proc. natn. Acad. Sci. U.S.A.* **77**, 649-652 (1980).
20. Otten, U. & Lorez, H. P. *Neurosci. Lett.* (in the press).
21. Ross, M., Löfstrand, S., Gorin, P. D., Johnson, E. M. & Schwartz, J. P. *J. Neuroscience* **1**, 1304-1311 (1981).
22. Mayer, N., Lembeck, F., Goedert, M. & Otten, U. *Neurosci. Lett.* **29**, 47-52 (1982).
23. Levi, A., Schechter, Y., Neufeld, E. J. & Schlessinger, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3469-3473 (1980).
24. Königsmark, B. W. in *Contemporary Research Methods in Neuroanatomy* (eds Nauta, J. H. & Ebesson, S. O. E.) 315-340 (Springer, New York, 1970).
25. Sternberger, L. A. *Immunocytochemistry* **246** (Prentice-Hall, Englewood Cliffs, New Jersey, 1974).
26. Redmann, G. *Microscopy* **33**, 102 (1972).
27. Mroz, E. A., Brownstein, M. J. & Leeman, S. E. in *Substance P* (eds von Euler, U. S. & Pernow, B.) 147-155 (Raven, New York, 1976).

Table 2 Substance P-immunoreactive neuronal cell bodies in lumbar dorsal root ganglia

Treatment	No. of ganglionic profiles evaluated	Area of ganglionic profiles evaluated (mm ²)	Substance P-like cells per 0.1 mm ² ganglionic profile area
a Control	18.6 ± 1.0	3.6 ± 0.1	18.9 ± 0.9 100%
b NGF	19.4 ± 1.5	4.6 ± 0.5	19.5 ± 1.1 104%
c Capsaicin	14.6 ± 0.4	2.2 ± 0.1	4.3 ± 0.3 23%
d NGF Capsaicin	19.2 ± 2.4	4.3 ± 0.5	12.5 ± 1.2 67%

Values indicate the means ± s.e.m. of five animals. Final data (see last column) were analysed by Mann-Whitney test; $2P > 0.05$ for a : b, < 0.01 for a : c, a : d, b : c, b : d and c : d. Two-day-old rats were injected s.c. with 20 µl of vehicle, NGF (0.5 µg g⁻¹), capsaicin (40 µg g⁻¹) or NGF and capsaicin. Capsaicin was injected once, NGF daily for 10 days. On day 14 the number of substance P-like neurones per ganglionic area was determined as described below. For immunohistochemistry of substance P, five animals per group anaesthetized with sodium pentobarbitone (60 µg g⁻¹, intraperitoneally) were perfused intracardially with 50 ml ice-cold 4% formaldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 15 min. The lumbar dorsal root ganglia were removed and transferred to this solution for an additional 3 h. They were then incubated for 4 days at 4°C in 0.1 M sodium phosphate buffer (pH 7.3) containing 20% (w/v) sucrose. From each rat five randomly chosen ganglia were embedded together and serially sectioned at 12 µm thickness in a cryostat at -15°C; in this way ~40 sections per rat with profiles of the ganglia were obtained. Five sections per rat were selected, each section usually containing three to four ganglionic profiles. Each ganglion was represented by at least two profiles in the section series. An interval of at least 72 µm was interposed between profiles of the same ganglion. For histochemistry of substance P-like immunoreactivity an antiserum raised in rabbits with a substance P carboxidiimide keyhole limpet haemocyanin conjugate (Immuno Nuclear Corp.) was used. Sections were stained by the peroxidase-antiperoxidase technique²⁵ following incubation with the primary antiserum diluted 1 : 300 for 1 h at 37°C. Staining specificity was established by absence of staining in sections run in parallel following incubation with the antiserum absorbed with substance P (40 mg ml⁻¹ undiluted serum) and following incubation with preimmune serum. In the ganglionic profiles of the five sections per rat, the cell bodies showing substance P immunoreactivity were counted by phase contrast microscopy using a ×25 objective. The area of the profiles was then measured using a ×16 objective and the manual optical picture analyzer MOP/AM 0.1 Kontron (ref. 26). From these data the number of substances P-immunoreactive cell bodies per 0.1 mm² ganglionic section area was determined.

First visualization of glutamate and GABA in neurones by immunocytochemistry

Jon Storm-Mathisen, Alfhild Kristine Leknes*, Anna Torbjørg Bore, Jorunn Line Vaaland, Paul Edminson†, Finn-Mogens Š. Haug & Ole Petter Ottersen

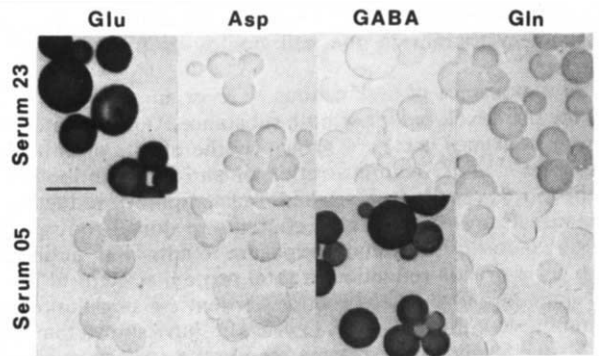
Anatomical Institute, University of Oslo, Karl Johansgt. 47, Oslo 1, Norway

†Paediatric Research Institute, University of Oslo, Rikshospitalet, Oslo 1, Norway

Immunocytochemical methods for peptides and serotonin have greatly advanced the study of neurones in which these substances are likely to be transmitters^{1,2}. Such direct techniques have not so far been available for the amino acid transmitter candidates. We report here the selective immunocytochemical visualization of the putative transmitters glutamate (Glu) and γ-aminobutyrate (GABA) by the use of antibodies raised against the amino acids coupled to bovine serum albumin (BSA) with glutaraldehyde (GA). The tissue localizations of Glu-like and GABA-like immunoreactivities (Glu-LI and GABA-LI) matched those of specific uptake sites for Glu and GABA, and, in the case of GABA-LI, also that of the specific marker enzyme glutamic acid decarboxylase (GAD). Thus, GABA-LI was located in what are believed to be GABAergic inhibitory neurones, whereas Glu-LI was concentrated in excitatory, possibly glutamatergic neurones. Preliminary electron microscopic observations suggest that the transmitter amino acids are significantly concentrated in synaptic vesicles.

*Deceased.

Fig. 1 Specificity of antisera tested by staining 'antigen'-bearing Sepharose gel beads by the peroxidase-antiperoxidase (PAP) method³. Supernatants (1,000g) of water homogenates of rat cerebral cortex, including the hippocampus, were dialysed against tap water followed by 0.1 M NaHCO₃ containing 0.5 M NaCl, after which free amino acids were not detectable (<20 pmol ml⁻¹, Rank-Hilger Chromospec amino acid analyser, post-column o-phthalaldehyde fluorescence). The cloudy extract was reacted with Sepharose 4B CNBr (Pharmacia). About 2 mg of polypeptide with 1.3 µmol of lysine was bound per ml gel (amino acid analysis as above after hydrolysis in 6M HCl). Amino acids (10 µmol and ³H-tracer per 0.5 ml packed gel in a total volume of 0.7 ml with 10 µmol sodium phosphate buffer pH 7.4) were fixed to the polypeptide-bearing gel beads by GA (2.5 mg). Only distilled GA (A₂₃₅/A₂₈₀ < 0.2)²⁰ was used in the present study. After washing, free aldehyde groups were reacted with 1 M ethanolamine. The amounts of amino acids bound were estimated from the proportions of tracer fixed (Table 1). The gel beads were suspended in 70% ethanol and stuck on to pre-gelatinized microtitre plates, to facilitate processing. The beads were incubated overnight with the primary sera. All sera were diluted in 0.1 M Tris-HCl buffer pH 7.4, containing 0.3 M NaCl, 1% normal sheep serum, 0.02% NaN₃ and 0.1% Triton X-100. Link antiserum (obtained by immunizing a sheep with purified rabbit IgG) was diluted 1:10, PAP complex (Miles-Yeda) was diluted 1:100. After development (diaminobenzidine and H₂O₂), the wells were filled with glycerol and sealed with transparent tape. Scale bar, 100 µm.



Glu or GABA (100 µmol) was conjugated to BSA (12 mg) by GA (100 µmol) in sodium phosphate buffer (2 ml 0.1 M, pH 7.4), and the product was dialysed and gel filtered. About 0.4 µmol of amino acid was bound per mg of native BSA. The BSA conjugates mixed with adjuvant were injected intracutaneously on the necks of rabbits. The crude sera, absorbed with BSA and tested by double diffusion in agarose gel, showed precipitation lines against the antigen used for immunization, but not against the other antigen or similar conjugates of BSA with aspartate, glutamine or glycine.

A method was devised for testing the antisera in conditions closely mimicking those of the immunocytochemical procedure³. Sepharose gel beads bearing amino acids fixed to brain protein extract with GA were immobilized in microtitre plates and processed like tissue sections (Fig. 1). The crude sera produced rather unselective staining of beads bearing various amino acids, and were therefore purified through a sequence of columns of Sepharose beads (S), bearing BSA or amino acids fixed to BSA by GA. The GABA antiserum 05 showed good selectivity after passage through BSA-S followed by Glu-GA-BSA-S (Fig. 1, Table 1). The cross-reactivity with leucine is probably unimportant, as the concentration of free Leu in brain is low, but cross-reactivity with taurine would interfere if the local concentration of Tau anywhere in the tissue is much higher than that of GABA. The Glu sera showed good selectivity after passage through the BSA-S and GABA-GA-BSA-S columns (Fig. 1, Table 1). After additional passage through columns of Gln-GA-BSA-S and Asp-GA-BSA-S there was no significant cross-reactivity with Gln or Asp (serum 13). Passing the sera through further columns of GA-histone-S, GA-BSA-S and GA-brain protein-S had no discernible effect on the staining of beads or sections.

The usefulness of the sera for cytochemistry in brain tissue was tested in the hippocampal formation, a region which contains well defined systems of excitatory and inhibitory neurones believed to use Glu and GABA as transmitters⁴⁻⁶. The axon terminals of these neurones are concentrated in separate zones. Thus, slices of fresh hippocampus cut into cold sucrose solution, fixed and stained with anti-Glu sera showed a striking laminar distribution of Glu-LI (Fig. 2), which was particularly concentrated in the terminal zones of excitatory systems where Glu is a strong transmitter candidate^{5,6}. As our antisera were unreactive with Leu-enkephalin and Met-enkephalin (Table 1), the staining in the mossy fibres and lateral perforant path terminal zones is not due to cross-reactivity with enkephalins, thought to be present in these systems^{7,8}. There was no staining in stratum pyramidale or stratum granulosum, where the terminals of inhibitory interneurones are concentrated⁴. Glu-LI seemed to be located in nerve terminals. This was particularly evident for the large mossy fibre boutons (Fig. 3a). Electron microscopy showed that these contained densely stained particles, consistent with a vesicular location of Glu (Fig. 3b). This is in

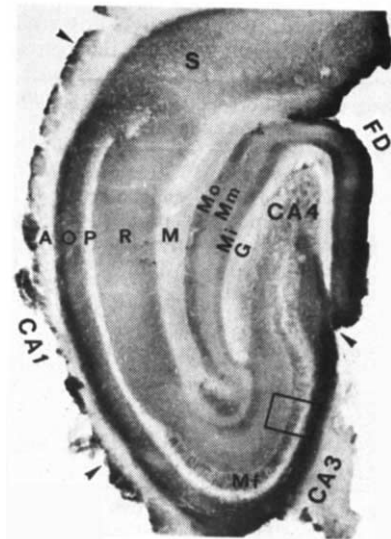


Fig. 2 Glu-LI is high in target zones (Mf, Mi, Mo, O, R) of certain excitatory afferents in the hippocampal formation. Regions (borders marked by ▼): CA1-4, subfields of hippocampus; FD, fascia dentata; S, subiculum. Zones: A, alveus; Mf, mossy fibre layer (target of axons from granular cells in FD); Mi, inner zone of molecular layer of FD (targets of pyramidal cell axons from CA4); Mm and Mo, zones of molecular layers in FD and CA3 (targets of medial and lateral parts of the perforant path); M, stratum lacunosum-moleculare of CA1; O and R, stratum oriens and stratum radiatum (targets of pyramidal cell axons from CA3, including Schaffer collaterals); P and G, stratum pyramidale and stratum granulosum (targets of inhibitory basket cells). Frame indicates position of area shown in Fig. 3a.

Methods: Slices were fixed (5% GA in 0.1 M sodium phosphate buffer pH 7.4, 25 °C, 30 min) after cutting (200 µm, Sorvall TC2), in cold phosphate-buffered (5 mM, pH 7.4) sucrose (0.3 M). The slices were treated with 1 M ethanolamine (adjusted to pH 7.4 with HCl in 0.1 M phosphate buffer), then with graded ethanol to 100% and with 0.3% H₂O₂ in methanol (to remove lipids and endogenous peroxidase activity). The immunocytochemical processing was essentially as in Fig. 1, with the sections free floating. Serum 13, diluted 1:80. (The increased staining near the edge of the slice is not biologically significant.) Preimmune serum (1:50 dilution), or serum 13 passed through the Glu-GA-BSA-S column to remove specific antibody (1:80 final dilution), produced only a weak, diffuse staining. Antibody eluted from the column (at pH 2.0) reproduced the specific staining. Similar slices stained with anti-GABA antiserum 05 (1:50 dilution) showed an entirely different pattern: high staining intensity around cell bodies (P, G) and in the superficial zones (M as strong as Mo). Note that cell bodies do not show Glu-LI or GABA-LI (nor Glu or GABA uptake⁶) in such slices. Scale bar, 500 µm.

Table 1 Microdensitometry of stained antigen-bearing Sepharose gel beads (see Fig. 1) showing specificity of sera used for Figs 2 and 3

Compound fixed to brain protein	$\mu\text{mol per ml gel}$	Glu antiserum 13	Glu antiserum 23	GABA antiserum 05
Glu	0.32	100.0 $\pm$ 3.5	100.0 $\pm$ 3.0	6.8 $\pm$ 0.8
Asp	0.20	3.3 $\pm$ 0.7	9.1 $\pm$ 0.5	5.2 $\pm$ 0.3
GABA	0.53	4.8 $\pm$ 0.6	2.8 $\pm$ 0.3	100.0 $\pm$ 6.0
Gln	0.42	4.6 $\pm$ 0.7	17.7 $\pm$ 1.4	7.7 $\pm$ 0.5
Gly	0.49	2.2 $\pm$ 0.5	1.0 $\pm$ 0.2	4.9 $\pm$ 0.5
Leu	0.55	2.4 $\pm$ 1.0	3.7 $\pm$ 0.4	16.1 $\pm$ 1.5
Pro	0.84	5.3 $\pm$ 0.6	2.2 $\pm$ 0.6	7.3 $\pm$ 0.5
Tau	0.54	3.5 $\pm$ 0.6	0.6 $\pm$ 0.4	19.6 $\pm$ 1.2
Ala	ND	0.6 $\pm$ 0.7	5.0 $\pm$ 0.7	8.0 $\pm$ 1.2
Leu-enkephalin	0.62	4.6 $\pm$ 0.7	2.2 $\pm$ 0.4	7.6 $\pm$ 0.5
Met-enkephalin	ND	3.0 $\pm$ 0.6	0.9 $\pm$ 0.2	6.4 $\pm$ 0.5
None	0.00	7.1 $\pm$ 0.7	3.0 $\pm$ 0.5	4.3 $\pm$ 0.3

Net absorbance is given as per cent of the maximum net absorbance for each serum (0.189 $\pm$ 0.007, 0.445 $\pm$ 0.013 and 0.358 $\pm$ 0.022 for sera 13, 23 and 05) and presented as mean $\pm$ s.e.m. of readings over 10–37 beads. Net absorbance was obtained by subtracting the average absorbance of unstained beads (0.013 $\pm$ 0.001). The net absorbance of Glu-bearing beads stained with preimmune serum diluted 1:50 corresponded to 6.9, 3.0 and 3.6% of the maximum net readings for sera 13, 23 and 05. The sera were obtained after four to nine immunizations over 3–21 months with 125–600 μg of antigen, mixed with incomplete, and later complete Freund's adjuvant. Purification through columns of Sepharose (S) conjugates: serum 13, BSA-S, GABA-GA-BSA-S (twice), Gln-GA-BSA-S, Asp-GA-BSA-S, GA-histone-S, brain extract-S, GA-brain extract-S; serum 23, BSA-S, GABA-GA-BSA-S, GA-histone-S (three times), GA-BSA-S, brain extract-S; serum 05, BSA-S, Glu-GA-BSA-S, GA-histone-S (three times), GA-BSA-S, brain extract-S. The sera were passed through the columns with 0.1 M Tris buffer pH 7.4 containing 0.3 M NaCl and 0.02% Na₂S₂O₃. The antibody-containing fractions (coinciding with the bulk of serum proteins) were concentrated by vacuum dialysis, and the final dilutions determined from the protein content (Lowry method). The columns were regenerated, usually by 0.1 M Gly buffer pH 2.0 containing 0.3 M NaCl (the Gln-GA-BSA-S column was regenerated at pH 12). Final dilutions: serum 13, 1:200; serum 23, 1:380; serum 05, 1:50. The absorbance of the beads was measured with a Zeiss projecting microscope and a Vitatron photometer (O. Grønnerød, H. A. Dahl, O. Vaage and L. Hermansen, unpublished), attached to a NORD 10 computer for averaging the differences of multiple paired readings over gel beads and background. The particles were only superficially stained, hence the absorbance was essentially independent of their size. The measuring spot covered only the central region of the beads, which had a single layer of staining (Fig. 1).

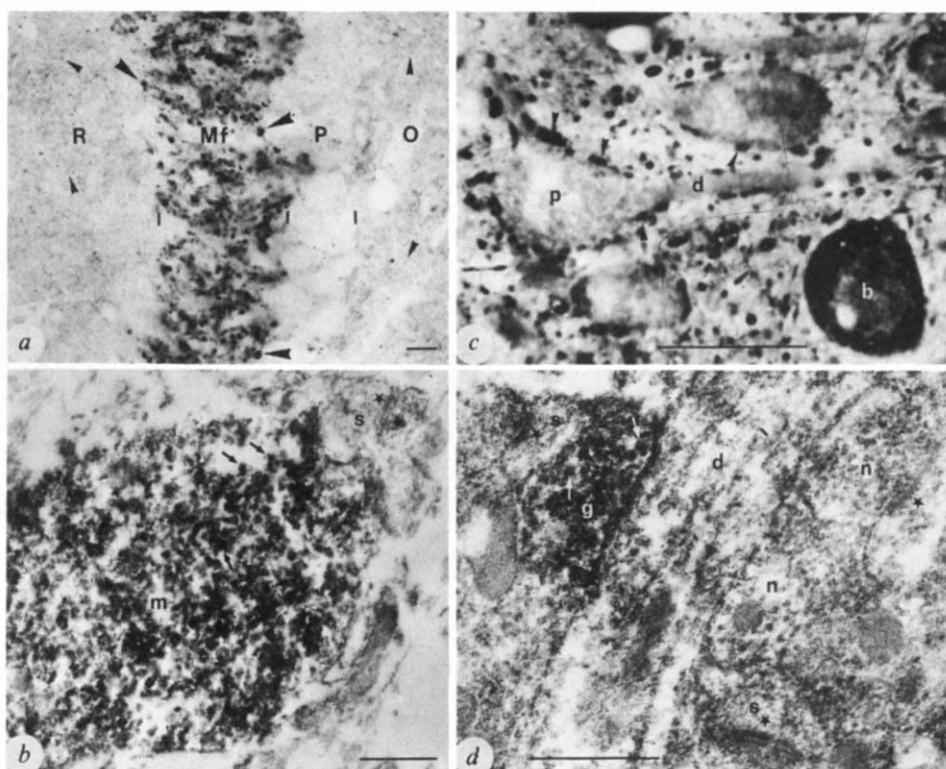
accord with the vesicular hypothesis for transmitter release, and with recent biochemical data which suggest that only a small proportion of the synaptosomal Glu is free in the cytosol⁹.

The pattern of Glu-LI described above (Fig. 2) corresponds well to that of high-affinity ³H-Glu uptake sites^{6,10} (alternative marker for putative glutamatergic boutons), with the exception that Glu-LI was relatively stronger in the mossy fibres and lateral perforant pathway. Incubation of the slices in Krebs' solution before fixation, in the conditions used to study high-affinity uptake autoradiographically, however, resulted in a pattern of Glu-LI identical to that of ³H-Glu uptake^{6,10}. Slices treated with either Krebs' or sucrose showed no Glu-LI, and no uptake of ³H-Glu, in cell bodies and larger dendrites. (The immunocytochemical preparations, as well as the autoradiograms⁶, detect activity only in the superficial few micrometres of the slice, where larger structures are cut open.)

In perfusion fixed tissue, on the other hand, perikarya and dendritic shafts were stained in pyramidal and granular cells, but not in non-pyramidal interneurons. The neuropil layers had high Glu-LI without a distinct laminar pattern, in agreement with biochemical analysis of freeze-dried sections¹¹, and apparently due to the staining of dendrites in addition to boutons. The *in vitro* procedure thus seems to remove Glu selectively from perikarya and dendrites, uncovering the localization of Glu-LI in certain excitatory boutons.

GABA-LI occurred in non-pyramidal perikarya (Fig. 3c) in perfusion fixed tissue, the location matching that of perikarya low in Glu-LI. The neuropil contained distinctly stained bouton-like structures (Fig. 3c), concentrated around the pyramidal and granular cell bodies and in the superficial cortical layers. The same pattern is seen for GAD-containing boutons^{4,12,13}. Electron microscopy revealed GABA-LI in boutons in contact with dendritic shafts, but not in boutons forming asymmetrical contacts with spines (Fig. 3d). Like Glu-LI, the GABA-LI seemed to be located in vesicles, suggesting that the low content of Glu and GABA in isolated vesicles¹⁴ may be due to leakage during preparation. However, immunocytochemical localization in organelles, particularly of compounds which are diffusible before fixation, must be

Fig. 3 a, Glu-LI in mossy fibre boutons (▼) and other boutons (▼) in CA3. Preparation and zones are as for Fig. 2 (frame), except that serum 13 was replaced by serum 23, which was diluted 1:380. b, Glu-LI in vesicular structures (↓) in a mossy fibre bouton (m) in contact with (*) an intra-terminal spine (s); electron micrograph of slice treated as in a, but serum diluted 1:150. c, GABA-LI visible in interneurons (b) and boutons (▼), but not in pyramidal cells (p), in subiculum. A Vibratome section (50 μm , Oxford) of perfusion fixed tissue (fixative as in Fig. 2) was osmicated, embedded in Araldite and resectioned (2 μm) after immunocytochemical staining with serum 05, diluted 1:50. d, GABA-LI in vesicular structures (↓) in a bouton (g) in contact with a dendritic shaft (d), but not in other boutons (n), some of which are in contact with dendritic spines (s) with dense postsynaptic specializations (*); same preparation as c, but CA1 zone R. For staining procedure and controls, see Fig. 2 legend. Controls showed no staining of nerve terminals. Scale bars: a, c, 20 μm ; b, d, 0.5 μm .



interpreted with caution. In slices, the distribution of GABA-LI was similar to that in perfusion fixed tissue, except that no perikarya were visible, and was the same as that of uptake sites for ^3H -GABA¹⁰.

We have recently begun to study other brain regions¹⁵. There is general agreement between GABA-LI and GAD immunocytochemistry¹⁶⁻¹⁸. In the cerebellum, for example, GABA-LI is present in terminals and perikarya of Golgi, basket, stellate and Purkinje cells, but not in granular cells. Glu-LI is generally low in perikarya with GABA-LI, and high in neurones believed to be glutamatergic, such as the cortical pyramidal cells¹⁹, but it is also present in cells not previously thought to be glutamatergic. Neuropil Glu-LI is high in regions with a rich supply of presumed glutamatergic afferents (for example, cortex, lateral septum, lateral geniculate, cerebellar molecular layer)¹⁹, but low in many areas with a strong GABAergic input (such as substantia nigra, globus pallidus,

cerebellar nuclei)¹⁹. Our approach should also allow the localization of other amino acids, for example, Asp, Gly and Tau, and provides the first localization of putative transmitter amino acids by direct immunocytochemical methods.

We thank S. Fossum for supplying the sheep antirabbit IgG, J. H. Johansen for the distilled glutaraldehyde, and A. Aakvaag, P. C. Emson, T. Eskeland, S. Fossum, A. Hamberger, G. A. R. Johnston, H. Karten, A. Matus, T. Michaelsen and E. Paus for discussions at various stages of this work; P. C. Emson helped improve the English. This work was supported by the Norwegian Research Council for Science and the Humanities (NAVF).

Note added in proof: Subsequent purification of the anti-GABA serum 05 through a column of Tau-GA-BSA-Sepharose reduced the cross-reactivity with Tau to 6.3% and that with Leu to 11.1% (see Table 1) without noticeable effects on the tissue localization of GABA-LI¹⁵.

Received 3 August; accepted 2 December 1982.

- Hököfelt, T., Johansson, O., Ljungdahl, Å., Lundberg, J. M. & Schultzberg, M. *Nature* **284**, 515-521 (1980).
- Pelletier, G., Steinbusch, H. W. M. & Verhofstad, A. A. J. *Nature* **293**, 71-72 (1981).
- Stenberger, L. A. *Immunocytochemistry* 2nd edn (Wiley, New York, 1979).
- Storm-Mathisen, J. *Prog. Neurobiol.* **8**, 119-181 (1977).
- Cotman, C. W. & Nadler, J. V. in *Glutamate: Transmitter in the Central Nervous System* (eds Roberts, P. J., Storm-Mathisen, J. & Johnston, G. A. R.) 117-154 (Wiley, Chichester, 1981).
- Storm-Mathisen, J. in *Glutamate: Transmitter in the Central Nervous System* (eds Roberts, P. J., Storm-Mathisen, J. & Johnston, G. A. R.) 89-115 (Wiley, Chichester, 1981).
- Gall, C., Brecha, N., Karten, H. J. & Chang, K.-J. *J. comp. Neurol.* **198**, 335-350 (1981).
- Fitzpatrick, D. & Johnson, R. P. *Neuroscience* **6**, 2485-2494 (1981).
- Kvamme, E. & Lenda, K. *Neurosci. Lett.* **25**, 193-198 (1981).
- Taxt, T. & Storm-Mathisen, J. *J. Physiol., Paris* **75**, 677-684 (1979).

- Nitsch, C. & Okada, Y. *J. Neurosci. Res.* **4**, 161-167 (1979).
- Barber, R. & Saito, K. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. & Tower, D. B.) 113-132 (Raven, New York, 1976).
- Ribak, C. E., Vaughn, J. E. & Saito, K. *Brain Res.* **140**, 315-332 (1978).
- Kontro, P., Marnela, K.-M. & Oja, S. S. *Brain Res.* **184**, 129-141 (1980).
- Ottersen, O. P. & Storm-Mathisen, J. in *Handbook of Chemical Neuroanatomy* (eds Björklund, A. & Hököfelt, T.) (Elsevier, Amsterdam, in the press).
- Roberts, E. in *GABA—Neurotransmitters* (eds Krogsgaard-Larsen, P., Scheel-Krüger, J. & Kofod, H.) 28-45 (Munksgaard, Copenhagen/Academic, New York, Nankodo, Tokyo, 1979).
- Pérez de la Mora, M. et al. *Neuroscience* **6**, 875-895 (1981).
- Oertel, W. H., Schmechel, D. E., Mugnaini, E., Tappaz, M. L. & Kopin, I. J. *Neuroscience* **6**, 2715-2735 (1981).
- Walaas, I. in *États Déficitaires Cérébraux Liés à l'Âge, Symp. Bel-Air VI* (ed. Tissot, R.) 47-82 (Georg, Librairie de l'Université, Geneva, 1980).
- Anderson, P. J. *J. Histochem. Cytochem.* **15**, 652-661 (1967).

Seizure-related brain damage induced by cholinergic agents

John W. Olney, Taisija de Gubareff & Joann Labruyere

Department of Psychiatry, Washington University School of Medicine, 4940 Audubon Avenue, St Louis, Missouri 63110, USA

Distinctive acute brain damage involving limbic and related brain regions develops in adult rats following sustained limbic seizures induced by systemic administration of kainic acid or dipiperidinoethane (DPE) or by intra-amygdaloid injection of kainic acid or folic acid¹⁻⁴. This seizure-brain damage (S-BD) syndrome is of particular interest because it tends to parallel the type of seizures and brain damage seen in human temporal lobe epilepsy^{5,6}. We have observed that DPE induces the S-BD syndrome by systemic but not intra-amygdaloid injection², whereas an oxidized DPE derivative which structurally resembles the cholinergic agonist oxotremorine is effective when injected into the amygdala⁷. Prompted by this finding, we injected known acetylcholine (ACh) agonists and cholinesterase (ChE) inhibitors into the rat amygdala and found that either class of agent reproduces this type of S-BD syndrome. These and related findings⁸ suggest that ACh mechanisms might have a more important role in human epilepsy and epileptic brain damage than has generally been appreciated.

The sustained limbic seizures that kainic acid is known to produce when administered to rats either systemically or into the amygdala are quite distinctive^{3,9-11}. They begin about 15 min after intra-amygdaloid injection with staring and facial grimaces then progress within 10-15 min to repetitive episodes of rearing on hind limbs with copious salivation and sustained clonus of forepaws and head; the rearing seizure episodes typically recur at less than 5-min intervals for more than 1 h (limbic status epilepticus)⁹. Animals that exhibit such repetitive seizure activity, whether induced by kainic acid, folic acid or DPE consistently display a disseminated pattern of acute brain damage involving several amygdaloid nuclei, the piriform,

entorhinal, insular and sensorimotor cortices, several thalamic nuclei, the hippocampus and lateral septum^{1,2,4,9,10,12}. The extent of the damage depends on the severity and duration of seizure activity. In addition, following direct injection into the amygdala, extensive local damage occurs about the injection site of kainic acid but not folic acid-injected rats^{1,3,4}. Thus, whereas both kainic and folic acid induce seizure-linked disseminated lesions, only kainic acid exerts a local neurodestructive action^{1,4,13}.

Oxotremorine, pilocarpine, carbachol, acetylcholine, nicotine, physostigmine and neostigmine or combinations of carbachol + atropine and neostigmine + acetylcholine were stereotactically injected into the adult rat amygdala (coordinates of Pellegrino *et al.*¹⁴ AP-0.2, L5.5, V9) under halothane anaesthesia using a Hamilton microsyringe and 28-gauge needle. The doses administered are given in Table 1. As external control, two agents previously found to have no S-BD potential when injected into the rat amygdala (DPE and *p*-aminobenzoylethylglutamate) were similarly injected, each at a single high dose exceeding the dose range used for cholinergic agents. All animals were observed behaviourally for 4 h after injection during which preconvulsive behaviours, convulsive episodes and time to onset of seizures were quantitatively evaluated and recorded. All rats were perfused transcardially with aldehyde fixatives either at 4 or 20 h for histopathological evaluation of the brains by previously published methods^{1,2,4,9,12,15}. Halothane anaesthesia was used because (unlike some anaesthetics, including barbiturates) it permits full development of S-BD phenomena and rats recover so quickly from halothane that behavioural monitoring is not hampered.

The number of animals studied and their responses to treatment are given in Table 1. Both ACh agonists and ChE inhibitors induced the S-BD syndrome, but the response to some agents, especially oxotremorine, was anomalous. Although one rat developed S-BD reaction following a low dose (20 nmol) of oxotremorine, no reaction developed in 27 other animals given doses ranging over 10-120 nmol. At a dose of 400 nmol, oxotremorine caused an S-BD syndrome in two out of four rats. To rule out the possibility that our sample of oxotremorine might be faulty the drug was administered subcutaneously (2 mg per kg) and elicited symptoms expected

Table 1 Incidence of S-BD syndrome in rats treated with cholinomimetics

<i>n</i>	Agents	Dose (nmol)	S-BD (%)*
Control agents			
6	Dipiperidinoethane	1,000	0
6	<i>p</i> -Aminobenzoyleglutamate†	1,000	0
ACh agonists			
6	Oxotremorine	10	0
5	Oxotremorine	20	20
5	Oxotremorine	30	0
5	Oxotremorine	40	0
7	Oxotremorine	60–120	0
4	Oxotremorine	400	50
4	Pilocarpine	50	0
6	Pilocarpine	100	33
6	Pilocarpine	150	17
3	Pilocarpine	200	33
6	Carbachol	12.5	67
7	Carbachol	25	100
6	Acetylcholine	50	0
6	Nicotine	20	0
ChE inhibitors			
4	Physostigmine	25	50
8	Physostigmine	50	87
5	Physostigmine	100	60
6	Neostigmine	1	0
7	Neostigmine	2	43
10	Neostigmine	3	80
4	Neostigmine	6	100
5	Neostigmine	12	80
6	Neostigmine	25	100
6	Neostigmine	50	100
Combined agents			
6	Acetylcholine + neostigmine	50 + 1	100
8	Acetylcholine + neostigmine	50 + 2	100
6	Carbachol + atropine	12.5 + 12.5	0
6	Carbachol + atropine	25 + 25	0

All agents were obtained from Sigma except DPE (Aldrich) and freshly prepared in sterile distilled water (pH adjusted to 7.2 ± 0.2) immediately before use. Concentrations were adjusted to permit administration in 1 μ l except for very high doses which were given in 3 μ l.

* Rats were considered as either positive or negative to the development of S-BD by the following criteria: positive; rats displaying status seizures (recurrent episodes at <5-min intervals for >1 h) and unequivocal tissue pathology. Negative; rats displaying no seizures or sporadic (non status) seizures and having either equivocal or no sign of tissue pathology. Coded histological slides were read by a trained neuropathologist.

† *p*-aminobenzoyleglutamate is an inactive component of the folic acid molecule.

of cholinergic substances (for example, tremor, ataxia, chromodacryorrhoea, tail rigidity, piloerection). The response to intra-amygdaloid pilocarpine was also rather anomalous in that a substantial per cent of animals were nonresponders at every dose tested. Carbachol was consistently effective in producing the S-BD syndrome and atropine, when injected together with carbachol, effectively prevented carbachol from inducing either seizures or brain damage.

Although the dose-response curve for physostigmine did not follow a linear progression, >50% of animals treated at each of three doses displayed unequivocal S-BD reactions (Fig. 1). Neostigmine was both the most potent and most consistently effective agent examined: at 1 nmol there was no response; at 2 nmol 3 out of 7 rats displayed mild S-BD reactions of markedly delayed onset (157 ± 34.8 min); at 3 nmol 8 out of 10 rats had moderate to severe S-BD reactions and at >3 nmol 20 of 21 displayed severe status epilepticus; seizures were rapid in onset (26.3 ± 2.4 min), recurred at less than 2-min intervals for more than 4 h and tissue damage according to the most

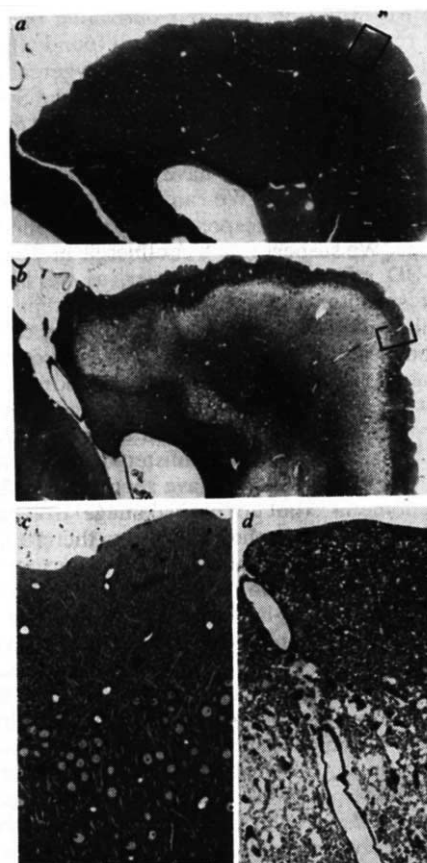


Fig. 1 Light micrographs of the caudal piriform cortex and amygdala of a control brain (a) injected with 1,000 nmol of DPE and experimental brain (b) injected with 25 nmol of physostigmine. Injections were made 24 h previously in the amygdala at a level ~2 mm rostral to that shown here. Evidence of acute necrosis of piriform cortical, amygdaloid and subicular neurones is shown by the rarified zones present in b and absent in a. Magnified views of boxed regions in a and b are shown in c and d. Histological sections 1 μ m thick were cut on a Dupont ultratome from Araldite-embedded blocks and stained with methylene blue/azure II as described elsewhere¹⁵ (a, b $\times 21$; c, d $\times 168$).

extreme pattern above described for kainic acid typically occurred. By co-injecting atropine (50 nmol) with neostigmine (2 nmol) we could delay onset and reduce the incidence of S-BD activity from an 80% control level to 30% ($n = 9$, data not shown). Thus, atropine blocks neostigmine less effectively than it blocks carbachol, a difference which may be related to the long duration of action of the neostigmine molecule. ACh (50 nmol), by itself, evoked neither seizures nor brain damage, but co-injecting ACh (50 nmol) with either 1 or 2 nmol neostigmine consistently caused a moderate to severe S-BD reaction with rapid seizure onset (27.4 ± 1.5 min). Nicotine was inactive in the amygdala at a dose (20 nmol) which reportedly¹⁶ induces a 'prostration' response when injected into brain stem nuclei containing nicotinic receptors.

Like folic acid⁴, both ACh agonists and ChE inhibitors induced damage in the amygdala which was less severe than that typically caused by kainic acid. This amygdaloid damage can be attributed to a seizure mechanism as amygdaloid neurones are part of the seizure circuit within which this type of seizure-linked damage takes place and, when injected into the striatum, folic acid induces the same degree of amygdaloid damage (plus other disseminated lesions) without causing any damage at the local injection site¹³. Moreover, intrastriatal neostigmine in high doses results in amygdaloid and other distant lesions without inducing local striatal damage (J.W.O. *et al.*, unpublished results).

Recently we tested an oxidized derivative of DPE which structurally resembles oxotremorine and found⁷ that by intra-amygdaloid injection it has S-BD effects whereas DPE itself does not. This supports the hypothesis² that the S-BD activity of subcutaneously administered DPE results from *in vivo* oxidation to an oxotremorine-like cholinergic agonist. Ironically, among the cholinomimetics we have now tested, oxotremorine has the least S-BD activity. We can suggest no explanation for this nor for the anomalous response pattern for pilocarpine and physostigmine. We suspect that a cholinergic mechanism mediates the S-BD effects noted, however, as we have recently found⁸ that subcutaneous injection of either pilocarpine or physostigmine, into rats pretreated with lithium chloride, consistently produces a kainic acid-like S-BD syndrome. Atropine protects against this lithium/cholinotoxic syndrome as it protects against the S-BD reaction to intra-amygdaloid carbachol. Furthermore, we now show that ACh itself consistently leads to an S-BD reaction when administered with low doses of neostigmine. Moreover, others have reported convulsions and kindling phenomena¹⁷ (but not brain damage) from intra-amygdaloid application of carbachol. It appears therefore, that activation of limbic ACh receptors, either by exogenous ACh agonists or by accumulation of endogenous ACh, is sufficient to induce a severe seizure-linked neurodegenerative syndrome.

However, we do not propose that persistent activity at ACh receptors is directly toxic to neurones bearing these receptors (although this possibility requires further study). Rather, we postulate that activation of ACh receptors results in sustained seizure activity which damages the brain by unknown mechanisms. As the endogenous excitatory transmitters, glutamate and aspartate, have the potential of destroying central neurones by a direct excitotoxic mechanism¹⁸, and these agents are probably released in excessive amounts at many synaptic loci in the course of sustained limbic seizures, it seems likely that these excitotoxins have a role in the brain damage associated with sustained limbic seizures (cholinergically or otherwise¹⁹⁻²¹ induced).

Cholinomimetics may be valuable tools for epilepsy research because they act by known mechanisms related to a specific central transmitter system and induce both seizures and seizure-related brain damage when injected either systemically⁸ or directly into brain. Such cholinergic mechanisms may be involved in the pathophysiology of brain damage associated with human epilepsy⁶.

This work was supported in part by grants from the Epilepsy Foundation of America and the USPHS (NS-09156, DA-00259, Research Career Scientist Award MH-38894 to J.W.O.).

Received 1 October; accepted 1 December 1982.

- Schwob, J. E., Fuller, T., Price, J. L. & Olney, J. W. *Neuroscience* **5**, 991-1014 (1980).
- Olney, J. W., Fuller, T. A., Collins, R. C. & Gubareff, T. *Brain Res.* **200**, 231-235 (1980).
- Ben Ari, Y., Tremblay, E., Ottersen, O. P. & Naquet, R. *Brain Res.* **165**, 362-365 (1979).
- Olney, J. W., Fuller, T. A. & de Gubareff, T. *Nature* **292**, 165-167 (1981).
- Menini, C., Meldrum, B. S., Riche, D., Silva-Comte, C. & Stutzmann, J. M. *Ann. Neurol.* **8**, 501-509 (1979).
- Corsellis, J. A. N., Meldrum, B. S. in *Greenfield's Neuropathology* 3rd edn (eds Blackwood, W. & Corsellis, J. A. N.) 771-795 (Arnold, Edinburgh, 1976).
- Olney, J. W., Collins, J. F. & de Gubareff, T. *Brain Res.* **249**, 195-197 (1982).
- Honchar, M. P., Olney, J. W. & Sherman, W. R. *Neurosci. Abstr.* **8**, 773 (1982).
- Collins, R. C., Lothman, E. W. & Olney, J. W. *Proc. UCLA, Symp. Status Epilepticus* (Raven, New York, 1981).
- Olney, J. W. in *Glutamate as a Neurotransmitter* (eds DiChiara, G. & Gessa, G. L.) 375-384 (Raven, New York, 1981).
- Fuller, T. A. & Olney, J. W. *Neurobehav. Tox. Teratol.* **3**, 355-361 (1981).
- Olney, J. W., Fuller, T. & deGubareff, T. *Brain Res.* **176**, 91-100 (1979).
- Olney, J. W., Fuller, T. A., de Gubareff, T. & Labryere, J. *Neurosci. Lett.* **25**, 185-191 (1981).
- Pellegrino, L. J., Pellegrino, A. S. & Cushman, A. J. *A Stereotaxic Atlas of the Rat Brain* 2nd edn (Plenum, New York, 1967).
- Olney, J. W. *J. Neuropharmacol. exp. Neurol.* **30**, 75-90 (1971).
- Aboud, L. G., Reynolds, D. T., Booth, H. & Bidlack, J. M. *Neurosci. Biobehav. Rev.* **5**, 479-486 (1981).
- Wasterlain, C. G., Masuoka, D. & Jonec, V. in *Kindling* (ed. Wada, J. A.) 315-334 (Raven, New York, 1981).
- Olney, J. W. in *Kainic Acid as a Tool in Neurobiology* (eds McGeer, E., Olney, J. W. & McGeer, P.) 95-122 (Raven, New York, 1978).
- Collins, R. C., Olney, J. W. & Lothman, E. R. in *Epilepsy* (ed. Ward, A. A. Jr) (Raven, New York, 1982).
- Sloviter, R. S. & Damiano, B. P. *Neurosci. Lett.* **24**, 279-284 (1981).
- Collins, R. C. & Olney, J. W. *Science* **218**, 177-179 (1982).

Ca²⁺ ions can affect intracellular pH in mammalian cardiac muscle

R. D. Vaughan-Jones*, W. J. Lederer† & D. A. Eisner‡

* Department of Pharmacology, University of Oxford, South Parks Road, Oxford OX1 3QT, UK

† Department of Physiology, University of Maryland School of Medicine, 660 West Redwood Street, Baltimore, Maryland 21201, USA

‡ Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK

Although intracellular pH (pH_i) has important effects on both the mechanical and electrical properties of cardiac muscle¹⁻³, the control of pH_i in the heart is still poorly understood. One important determinant of pH_i appears to be the transmembrane Na⁺ gradient^{4,5}. It has therefore been suggested that Na⁺-H⁺ exchange assists in the control of pH_i in heart as has been proposed for other excitable cells⁶⁻⁸. However, pH_i and the intracellular Ca²⁺ concentration ($[Ca^{2+}]_i$) are interdependent in a variety of tissues⁹⁻¹¹ and it has been shown recently that pH_i can affect $[Ca^{2+}]_i$ in cardiac muscle^{5,12}. As $[Ca^{2+}]_i$ in cardiac muscle is also strongly influenced by the transmembrane Na⁺ gradient⁵ it is possible that the apparent Na⁺-dependence of pH_i is secondary to changes in $[Ca^{2+}]_i$. Previous work in cardiac muscle has not been able to separate the effects of Na⁺-H⁺ exchange and $[Ca^{2+}]_i$ on pH_i (refs 4, 5). Here we demonstrate in cardiac muscle that an increase in $[Ca^{2+}]_i$ produces an intracellular acidification which cannot be ascribed to Na⁺-H⁺ exchange.

The experiments were performed on voltage-clamped sheep cardiac Purkinje fibres. Intracellular pH and intracellular Na⁺ activity (a_{Na}^i) were measured directly using ion-selective micro-electrodes (see Fig. 1C). Tension was also measured to give an estimate of changes in $[Ca^{2+}]_i$. The experiment of Fig. 1 shows the effect on pH_i of adding strophanthidin (10 μ M) to inhibit the Na⁺ pump. This procedure increases a_{Na}^i and, via a transmembrane Na⁺-Ca²⁺ exchange^{13,14}, increases $[Ca^{2+}]_i$ (refs 5, 15). The rise in a_{Na}^i was accompanied by an increase in twitch and tonic tension and the appearance of after-contractions indicating a rise in $[Ca^{2+}]_i$ (refs 16, 17). There was also a simultaneous acidification (0.1 pH units). Two hypotheses have been proposed for this acidification⁴: (1) the rise in a_{Na}^i acts on a transmembrane Na⁺-H⁺ exchange to produce a net influx of protons; (2) the rise in $[Ca^{2+}]_i$ displaces protons from common intracellular binding sites. Experiments of the type shown in Fig. 1 cannot distinguish between these two possibilities as both a_{Na}^i and $[Ca^{2+}]_i$ are increasing. Figure 2, however, shows that the distinction can be made if the external Ca²⁺ concentration, $[Ca^{2+}]_o$, is changed. In this preparation, after the Na⁺-K⁺ pump had been inhibited (K⁺-free solution), a reduction in $[Ca^{2+}]_o$ from 5 to 0.5 mM decreased $[Ca^{2+}]_i$, as evidenced by the decrease in all components of tension (Fig. 2B). Simultaneously, a_{Na}^i increased while pH_i became more alkaline. All these effects could be reversed by returning to 5 mM $[Ca^{2+}]_o$. The effect of $[Ca^{2+}]_o$ on a_{Na}^i has been attributed to Na⁺-Ca²⁺ exchange⁴. Figure 2 demonstrates that pH_i is also affected. In this case, however, the effect on pH_i cannot be ascribed to the changes of a_{Na}^i acting on a Na⁺-H⁺ exchange: these would be expected to alter pH_i in the opposite direction. On the other hand, the results are consistent with a model in which a rise in $[Ca^{2+}]_i$ leads to an acidification.

Although the results of Fig. 2 exclude a significant contribution from Na⁺-H⁺ exchange, they do not indicate whether the effects of calcium on pH_i are mediated directly via changes in $[Ca^{2+}]_o$ or are secondary to alterations of $[Ca^{2+}]_i$. In the experi-

ment of Fig. 3, inhibition of the Na^+ - K^+ pump again produced a slow intracellular acidification. Reduction in $[\text{Ca}^{2+}]_o$ from 5 to 0.5 mM produced a fall in tension and reduced the rate of acidification. However, when $[\text{Ca}^{2+}]_o$ was subsequently restored to 5 mM, there was a marked increase in resting tension, indicating a rise of $[\text{Ca}^{2+}]_i$, and this was accompanied by a rapid acidification. Reduction of $[\text{Ca}^{2+}]_o$ to 0.5 mM partly reversed these effects. The large contraction on restoring 5 mM $[\text{Ca}^{2+}]_o$ is expected as the previous exposure to low $[\text{Ca}^{2+}]_o$ should have increased a_{Na}^i (see Fig. 2). At a given $[\text{Ca}^{2+}]_o$, raising a_{Na}^i will increase Ca^{2+} entry via Na^+ - Ca^{2+} exchange. The fact that the acidification was faster during the second exposure to 5 mM $[\text{Ca}^{2+}]_o$ suggests that the effects of $[\text{Ca}^{2+}]_o$ on pH_i depend on

$[\text{Ca}^{2+}]_i$, and not merely on the level of $[\text{Ca}^{2+}]_o$. A related observation (not shown) is that changes in $[\text{Ca}^{2+}]_o$ have little effect on pH_i if the Na^+ pump is not inhibited and a_{Na}^i is low. This is expected as, when a_{Na}^i is low, Ca^{2+} influx is decreased¹⁴ and changes in $[\text{Ca}^{2+}]_o$ have only modest effects on $[\text{Ca}^{2+}]_i$ (refs 18, 19).

These results suggest that the acidification produced by increasing $[\text{Ca}^{2+}]_o$ is mediated by an increase in $[\text{Ca}^{2+}]_i$. In considering the mechanism of this effect, although we cannot exclude an action of $[\text{Ca}^{2+}]_i$ on transmembrane proton movements, a more attractive explanation is that raising $[\text{Ca}^{2+}]_i$ displaces protons from common intracellular buffer sites. This has been demonstrated in snail neurones⁹ where the Ca^{2+} - H^+ interaction occurs mainly at the mitochondria. The 0.15 pH acidification produced by increasing $[\text{Ca}^{2+}]_o$ (Fig. 2) is equivalent to an increase of ~ 5 mmol of protons per l of cytoplasm (assuming an intracellular proton buffering power of 35 mmol per pH unit²⁰). If these protons are displaced by Ca^{2+} from common buffer sites, the buffer must have an adequate capacity and this immediately excludes troponin, the main known cytoplasmic buffer²¹. A Ca^{2+} - H^+ interaction at the sarcoplasmic reticulum is a possibility. In skeletal muscle, for

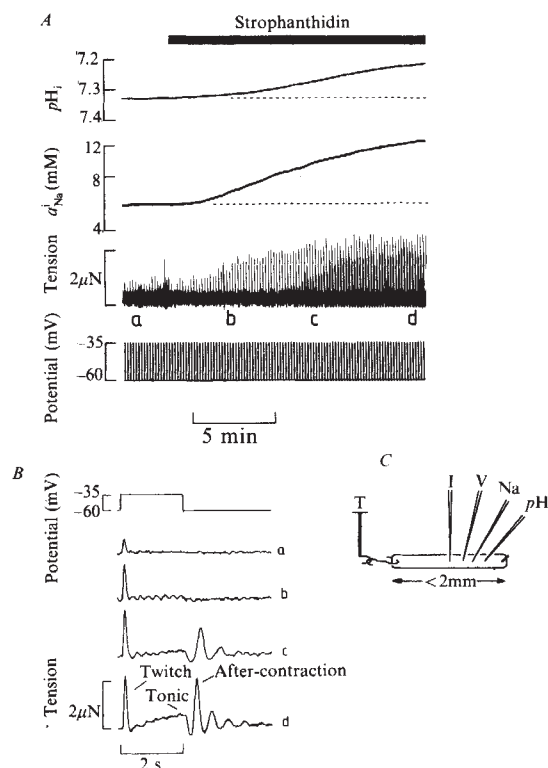


Fig. 1 Effects of Na^+ pump inhibition on pH_i , a_{Na}^i and muscle contraction. **A**, time course of onset of effects. Traces show, from top to bottom: pH_i , a_{Na}^i , tension and membrane potential. The solution protocol is shown above the record. Strophanthidin (10 μM) was applied for the period shown. Throughout the experiment the membrane potential was held at -60 mV and depolarizing voltage-clamp pulses of 2 s duration were applied to -35 mV at 0.1 Hz to elicit contractions. The pH_i and a_{Na}^i traces have been low-pass filtered (time constant 10 s). The tension trace was high-pass filtered (time constant 10 s) to remove d.c. changes in tension. The filtering allows accurate resolution of only the twitch magnitude. **B**, specimen records of contraction produced by the depolarizing voltage-clamp pulse shown at the top. **a-d** Refer to the corresponding points in **A**. These records have not been filtered and allow resolution of the twitch and tonic components of tension as well as the after-contraction; these components are identified on trace **d**. Each of the records has been signal-averaged ($n=8$). All the solutions contained 4 mM RbCl and 5 mM CaCl_2 . **C**, schematic diagram of the apparatus. T, tension transducer; I, current electrode; V, voltage electrode; Na^+ , Na^+ -selective microelectrode; pH , pH -selective microelectrode. Sheep cardiac Purkinje fibres were shortened to a length of 1–2 mm and attached to a tension transducer. A two-microelectrode voltage-clamp was used to control membrane potential. a_{Na}^i and pH_i were measured with recessed-tip pH - and Na^+ -selective glass microelectrodes²⁹. (For further experimental details see ref. 30.) The solutions used contained (in mM): NaCl, 145; MgCl_2 , 1; Tris-HCl (pH 7.4 at 37 °C), 10; glucose, 10 and were equilibrated with air. All solutions were K^+ -free and contained the concentrations of RbCl and CaCl_2 indicated in the relevant legend. Rb was used as a convenient K^+ substitute for the Na^+ pump³⁰.

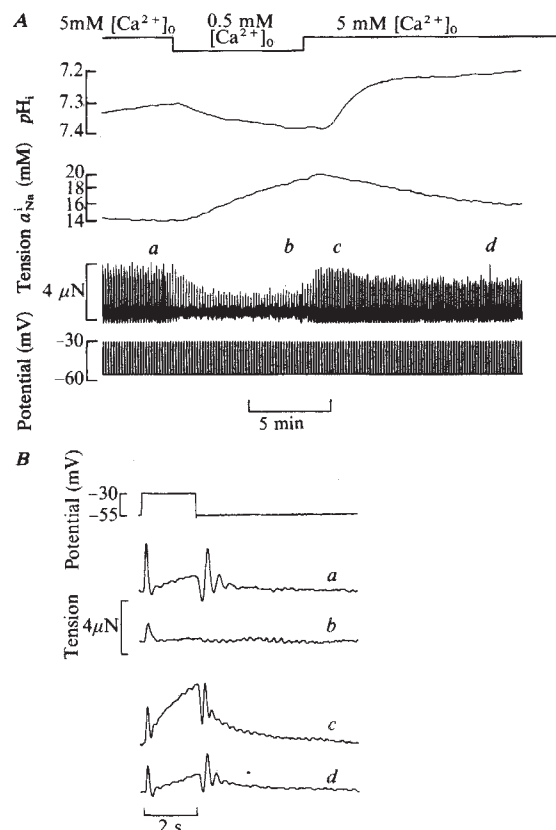


Fig. 2 The effects of changing the external Ca^{2+} concentration $[\text{Ca}^{2+}]_o$ on pH_i , a_{Na}^i and contraction. **A**, time course of effects. Traces show, from top to bottom: pH_i , a_{Na}^i , tension and membrane potential. The fibre had been exposed to a K^+ -free, Rb-free solution for 20 min to inhibit the Na^+ pump before the beginning of the record shown. At the times indicated at the top of the figure $[\text{Ca}^{2+}]_o$ was first reduced from 5 to 0.5 mM and then increased back to 5 mM. Throughout the experiment the membrane potential was held at -55 mV and depolarizing voltage-clamp pulses (2 s duration) to -30 mV were applied at 0.1 Hz. The pH_i and a_{Na}^i records have been low-pass filtered (time constant 10 s) and the tension trace was high-pass filtered (time constant 10 s) to remove d.c. changes in tension. **B**, specimen records of contraction produced by the depolarizing voltage-clamp pulse shown at the top. **a-d** Refer to the corresponding points in **A**. Each of the records has been signal-averaged ($n=8$). The records were not filtered.

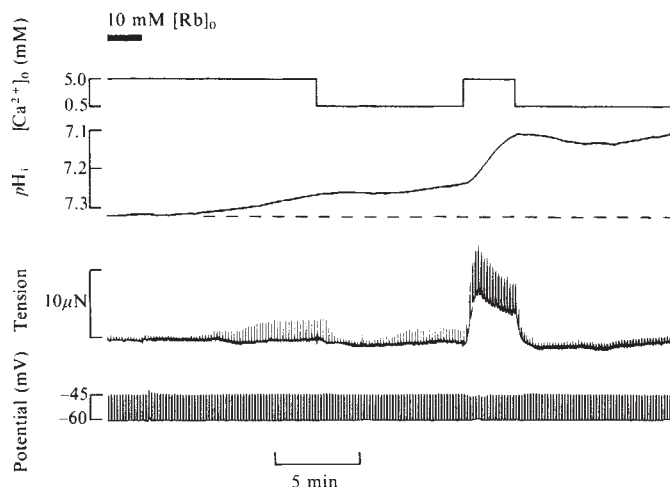


Fig. 3 The effects of changing the external Ca^{2+} concentration on pH_i and tension. Traces show, from top to bottom: pH_i , tension and membrane potential. The solution protocol is shown at the top of the figure. The preparation was initially exposed to 10 mM Rb and, at the time shown, Rb was reduced to zero to inhibit the Na^+ pump. Then $[\text{Ca}^{2+}]_o$ was reduced to 0.5 mM and then increased back to 5 mM before finally reducing it to 0.5 mM again. Throughout the experiment the membrane potential was held at -60 mV and depolarizing voltage-clamp pulses to -45 mV (2 s duration) were applied at 0.1 Hz. The pH_i trace has been low-pass filtered (time constant 10 s). The tension trace has not been filtered and therefore shows accurately changes in twitch and tonic tension.

example, acidification releases Ca^{2+} ions from the sarcoplasmic reticulum²² and the reverse effect (calcium-releasing protons) could explain the present results. Finally, a Ca^{2+} - H^+ exchange may occur at the mitochondria⁹. This would require the uptake of 2.5 or 5 mmol Ca^{2+} ions per l of cytoplasm, depending on whether one or two H^+ ions exchange for each Ca^{2+} ion²³. The density of mitochondria in a Purkinje fibre²⁴ and values for their Ca^{2+} -binding capacity²³ suggest that they could provide sufficient buffering. Thus, there are sufficient intracellular sites where Ca^{2+} could displace H^+ to produce the observed changes in pH_i . It is now of interest to compare the magnitude of the changes in proton and Na^+ concentration when $[\text{Ca}^{2+}]_o$ is increased (Fig. 2). The fall in a_{Na}^i of ~ 5 mM is equivalent to a reduction in Na^+ concentration of 6.7 mM (assuming a Na^+ activity coefficient of 0.75). If this occurs by a Na^+ - Ca^{2+} exchange which exchanges two or three Na^+ ions per Ca^{2+} ion, a rise in total Ca^{2+} of ~ 2 –3.5 mM would be expected. This is of the same order of magnitude as the increase estimated above from the change in pH_i . The present results therefore agree reasonably well with a model in which raising $[\text{Ca}^{2+}]_o$ decreases a_{Na}^i via Na^+ - Ca^{2+} exchange and decreases pH_i via an increase in $[\text{Ca}^{2+}]_i$, displacing protons.

The effects of $[\text{Ca}^{2+}]_i$ on pH_i have important consequences for experiments in which the influence of $[\text{Ca}^{2+}]_i$ on various cellular systems is examined. Two clear examples in heart are contraction and the control of intercellular electrical coupling, as both are affected by $[\text{Ca}^{2+}]_i$ and pH_i (refs 25–27). The influence of $[\text{Ca}^{2+}]_i$ on pH_i also has implications for the regulation of intracellular pH in heart. It has been suggested that, in different conditions, either Na^+ - H^+ or Cl^- - HCO_3^- exchange mechanisms may control pH_i (refs 4, 28). The present work suggests that $[\text{Ca}^{2+}]_i$ can also influence pH_i . As Na^+ - Ca^{2+} exchange affects $[\text{Ca}^{2+}]_i$, the regulation of pH_i becomes indirectly dependent on the transmembrane Na^+ gradient. In other tissues as well as cardiac muscle, the dependence of pH_i on sodium has often been taken as evidence for the control of pH_i by a Na^+ - H^+ exchange mechanism. Although we certainly do not exclude the existence of such an exchange in the heart, we emphasize that it does not underlie the apparent Na^+ -dependence of pH_i observed in the present work.

We thank the following for grant support: the British Heart Foundation, the MRC and the Wellcome Trust (D.A.E.); March of Dimes Birth Defects Foundation, the NIH (HL-25675) and the American Heart Association and its Maryland Affiliate (W.J.L.); and the MRC (R.D.V.-J).

Received 20 August; accepted 2 December 1982.

- Gaskell, W. H. *J. Physiol., Lond.* **3**, 48–75 (1880).
- Poole-Wilson, P. A. *J. molec. cell. Cardiol.* **10**, 511–526 (1978).
- Marrannes, R., de Hemptinne, A. & Leusen, I. *Pflügers Arch. ges. Physiol.* **389**, 199–209 (1981).
- Deitmer, J. W. & Ellis, D. *J. Physiol., Lond.* **304**, 471–488 (1980).
- Bers, D. M. & Ellis, D. *Pflügers Arch. ges. Physiol.* **393**, 171–178 (1982).
- Thomas, R. C. *J. Physiol., Lond.* **273**, 317–338 (1977).
- Aickin, C. C. & Thomas, R. C. *J. Physiol., Lond.* **273**, 295–316 (1977).
- Roos, A. & Boron, W. F. *Physiol. Rev.* **61**, 296–434 (1981).
- Meech, R. W. & Thomas, R. C. *J. Physiol., Lond.* **298**, 111–129 (1980).
- Baker, P. F. & Honerjager, P. *Nature* **273**, 160–161 (1978).
- Lea, T. J. & Ashley, C. C. *Nature* **275**, 236–238 (1978).
- Hess, P. & Weingart, R. *J. Physiol., Lond.* **307**, 60P (1980).
- Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, R. A. *J. Physiol., Lond.* **200**, 295–316 (1969).
- Glitsch, H. G., Reuter, H. & Scholz, H. *J. Physiol., Lond.* **209**, 25–43 (1970).
- Sheu, S.-S. & Fozzard, H. J. *gen. Physiol.* **80**, 325–351 (1982).
- Kass, R. S., Lederer, W. J., Tsien, R. W. & Weingart, R. *J. Physiol., Lond.* **281**, 187–208 (1978).
- Eisner, D. A. & Lederer, W. J. *J. Physiol., Lond.* **294**, 255–277 (1979).
- Marban, E., Rink, T. J., Tsien, R. W. & Tsien, R. Y. *Nature* **286**, 845–850 (1980).
- Coray, A., Fry, C. H., Hess, P., McGuigan, J. S. & Weingart, R. *J. Physiol., Lond.* **305**, 60–61P (1980).
- Ellis, D. & Thomas, R. C. *J. Physiol., Lond.* **262**, 755–771 (1976).
- Katz, A. M. *Physiol. Rev.* **50**, 63–158 (1970).
- Lea, T. J. & Ashley, C. C. *J. Membrane Biol.* **61**, 115–125 (1981).
- Carafoli, E. & Crompton, M. *Curr. Topics Membrane Transp.* **10**, 151–214 (1978).
- Mobley, B. A. & Page, E. *J. Physiol., Lond.* **220**, 547–563 (1972).
- Fabiato, A. & Fabiato, F. *J. Physiol., Lond.* **276**, 233–255 (1978).
- Dahl, G. & Isenberg, G. *J. Membrane Biol.* **53**, 63–75 (1980).
- Reber, W. R. & Weingart, R. *J. Physiol., Lond.* **328**, 87–104 (1982).
- Vaughan-Jones, R. D. *Phil. Trans. R. Soc.* **299**, 537–548 (1982).
- Thomas, R. C. *Ion-sensitive Intracellular Microelectrodes* (Academic, London, 1978).
- Eisner, D. A., Lederer, W. J. & Vaughan-Jones, R. D. *J. Physiol., Lond.* **317**, 163–187 (1981).

Release of somatostatin-28(1–12) from rat hypothalamus *in vitro*

C. Bakhit, R. Benoit & F. E. Bloom

Arthur V. Davis Center for Behavioral Neurobiology,
The Salk Institute, San Diego, California 92138, USA

Following the discovery of the growth hormone release-inhibiting factor somatostatin from extracts of ovine hypothalamus¹, an N-terminally extended somatostatin of 28 amino acids has been identified in mammalian tissue^{2–6}. The original peptide, somatostatin-14 (SS14), corresponds to the C-terminus of somatostatin-28 (SS28). Both SS28 and SS14 have biological activity^{3,7–9}, occur in several rat brain regions^{10–15}, are present in cell bodies and nerve terminals^{11–14,16} and can be released *in vitro* upon depolarization in a calcium-dependent manner^{16–19}. Further, high-affinity binding sites were described for SS14, which also bind SS28 (refs 20–23). Recently, a dodecapeptide which corresponds to the N-terminus of somatostatin-28, somatostatin-28(1–12), has been characterized in rat hypothalamus²⁴. Radioimmunological²⁵ and immunohistochemical¹³ studies have indicated the presence of SS28(1–12)-like immunoreactivity in several cortical and sub-cortical regions of the rat brain. This peptide was found to be unevenly distributed with the highest concentration in the hypothalamus, and preferentially localized to dendritic and axonal processes and terminals. These observations suggest that SS28(1–12) may be a neurotransmitter. In this study, we describe a calcium-dependent release of a SS28(1–12)-like peptide from hypothalamic slices *in vitro*. This finding supports a neurotransmitter function for this peptide.

Male Sprague-Dawley rats weighing 200–220 g were used in this study. Hypothalamic fragments weighing approximately 40 mg each, were rapidly dissected from 12 rat brains and

cross-cut at 250 μm intervals using a McIlwain tissue chopper. The resulting slices were washed three times with a modified Krebs-bicarbonate solution containing the following: NaCl 127 mM; KCl 3.83 mM; CaCl_2 1.8 mM; KH_2PO_4 1.18 mM; MgSO_4 1.18 mM; NaHCO_3 20 mM; D-glucose 2 g l^{-1} ; bovine serum albumin (Miles) 0.1%; bacitracin (Sigma) 30 $\mu\text{g ml}^{-1}$. The medium was gassed with an oxygen/carbon dioxide (95:5, v/v) mixture. The final pH was 7.4. For certain manipulations, the above medium was modified in three ways: (1) 50 mM KCl substituted in part for NaCl (isomolar replacement), (2) Ca^{2+} -free Krebs media (CFM) + 1 mM CoCl_2 , (3) CFM containing 50 mM KCl and 1 mM CoCl_2 (CFM-50 mM KCl). Tissue slices from six hypothalami suspended in Krebs-bicarbonate medium were placed in a superfusion chamber made up of a 1-inch diameter 'Easy Pressure' filter holder with a 0.45 μm Metrical membrane filter (Gelman). The Krebs-bicarbonate was applied to the superfusion chamber at a rate of 400 $\mu\text{l min}^{-1}$ using a 5 ml syringe mounted on a delivery apparatus. All experiments were performed at 24 $^\circ\text{C}$. After an initial wash period of 10 min, superfusate samples were collected at 2 min intervals, immediately boiled for 15 min and kept frozen until assayed. SS28(1-12)-like immunoreactivity [SS28(1-12)-LI] was measured using a specific antiserum which detects the free C-terminus of SS28(1-12) but not SS14 or SS28. At the end of each experiment, the hypothalamic slices were extracted in 4 ml of 1M acetic acid, boiled, homogenized, centrifuged and the resulting supernatant freeze-dried for subsequent radioimmunoassay^{14,24}.

During the initial 10-min wash-out period, rapid release of SS28(1-12)-LI was observed, after which the spontaneous release of SS28(1-12)-LI decreased to below 0.1% of total tissue content per min. In some instances, the concentration of spontaneously released SS28(1-12)-LI in these wash-out samples was less than that which could be accurately measured by our radioimmunoassay (RIA) (sensitivity of assay, 25 fmol per tube). When the superfusion medium was changed to a high K^+ medium (K^+ 50 mM), a large increase in SS28(1-12)-LI release was observed (Fig. 1). During this stimulation period, which lasted 8 min, the rate of SS28(1-12)-LI release was several-fold higher than in the spontaneous release period. Approximately 1.5% tissue content of SS28(1-12)-LI was

released as a result of K^+ ion stimulation. When the superfusion medium was changed to a calcium-free medium containing Co^{2+} , which inhibits Ca^{2+} actions during synaptic release of transmitter²⁶, basal release levels of SS28(1-12)-LI were observed and no further release by high K^+ was elicited (Fig. 1).

It is conceivable that SS28(1-12)-LI as measured in our assay might be a breakdown product of SS28, which is initially released into the medium and subsequently degraded, rather than direct release of SS28(1-12)-LI. To test this possibility, 50 pmol of SS28 were added to the superfusion medium and passed, in one case, through the layer of tissue slices from three hypothalami, and in another, through a perfusion chamber that had no tissue slices, for 5 min. There was no detectable increase in SS28(1-12)-like material above basal or background levels. This finding indicates that degradation of SS28 does not occur to any significant extent under our experimental conditions.

To verify if the material measured in the perfusate sample contains the SS28(1-12) antigenic determinant, pooled samples collected from spontaneous and high K^+ stimulated release were centrifuged, the supernatant lyophilized and the residue dissolved in 30% acetic acid and fractionated on a Sephadex G-50 (fine) column. Fractions eluted from the column and corresponding to peptides between 600 and 6,000 molecular weight were combined, lyophilized and the dry material from the spontaneous and stimulated release were diluted in radioimmunoassay buffer, tested in serial dilutions and compared with a SS28(1-12) synthetic standard. Figure 2 shows parallel displacement of labelled Tyr-SS28(1-12) bound to the antibody by the material from spontaneous and stimulated release, and by synthetic SS28(1-12). Thus, the data suggest that the immunoreactive material measured in our release experiment contains the SS28(1-12) structure.

These results demonstrate that somatostatin-28(1-12)-LI can be released from rat hypothalamic slices by a depolarizing concentration of K^+ *in vitro*. The K^+ -evoked release is Ca^{2+} -dependent. Our present results for the release of SS28(1-12)-LI are similar to those previously reported for SS14 and SS28^{16,17} and other centrally acting neuropeptides. The data suggest that SS28(1-12)-LI may normally be released in the central nervous system to regulate in the transmission of neural information.

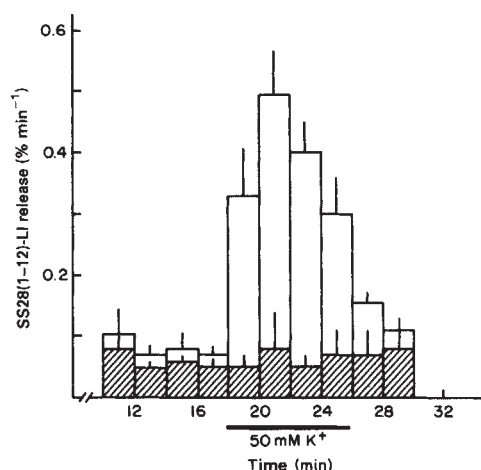


Fig. 1 Release of somatostatin 28(1-12)-LI from hypothalamic slices. The open columns represent SS28(1-12)-LI in the presence of calcium ions and the stippled columns represent SS28(1-12)-LI when 1 mM CoCl_2 was substituted for Ca^{2+} in the perfusion medium. Results are expressed as percentage of tissue content measured in tissue slices at the end of the experiment. Values are mean $\pm$ s.e.m. of three experiments. The mean values only included experiments in which basal release SS28(1-12)-LI was measurable. The tissue chamber void volume was approximately 0.5 ml.

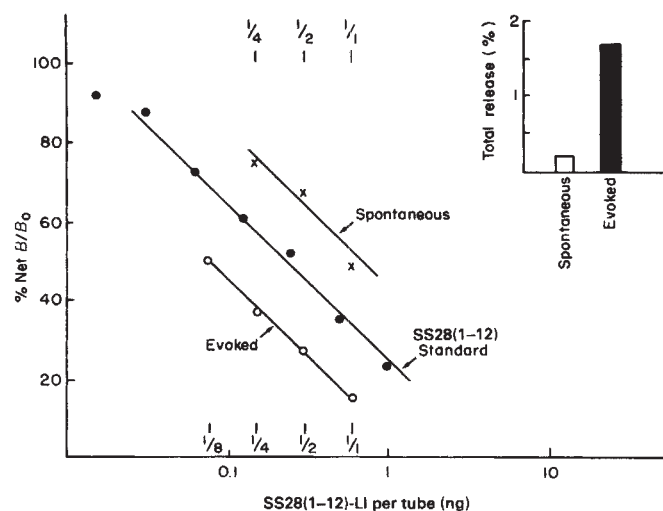


Fig. 2 Radioimmunoassay competition curves showing parallel displacement of iodinated Tyr-SS28(1-12) bound to antiserum by pooled spontaneous release samples, pooled K^+ -evoked release samples and synthetic SS28(1-12). The position of the lines was calculated by linear regression.

We thank Dr Nicholas Ling for providing the peptides required for the radioimmunoassays, Barbara Alford for technical assistance, and Nancy Callahan for typing the manuscript. This research was funded by grants AA 07273 and NIADDK 26741 and Canadian MRC Fellowship to R.B.

Received 10 September, accepted 24 November 1982.

1. Brazeau, P. *et al. Science* **179**, 77–79 (1973).
2. Pradayrol, L., Jornvall, H., Mutt, V. & Ribet, A. *FEBS Lett.* **109**, 55–58 (1980).
3. Schally, A. V. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 4489–4493 (1980).
4. Esch, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 6827–6831 (1980).
5. Spiess, J., Villareal, J. & Vale, W. *Biochemistry* **20**, 1982–1988 (1981).
6. Bohlen, P., Brazeau, P., Esch, F., Ling, N. & Guillemin, R. *Regulat. Peptides* **2**, 359–369 (1981).
7. Renaud, L. P., Martin, J. B. & Brazeau, P. *Nature* **255**, 233–235 (1975).
8. Ioffe, S., Havlicek, V., Friesen, H. & Chernick, V. *Brain Res.* **153**, 414–418 (1978).
9. Brazeau, P. *et al. C.R. hebdom. Séanc. Acad. Sci., Paris* **290**, 1369–1371 (1980).
10. Brownstein, M., Arimura, A., Sato, H., Schally, A. V. & Kizer, J. S. *Endocrinology* **96**, 1456–1461 (1975).
11. Hokfelt, H. *et al. Neuroscience* **1**, 131–136 (1976).
12. Epelbaum, J., Brazeau, P., Tsang, D., Brawer, J. & Martin, J. B. *Brain Res.* **126**, 309–323 (1977).
13. Morrison, J. H., Benoit, R., Magistretti, P. J. & Bloom, F. E. *Brain Res.* (in the press).
14. Benoit, R., Ling, N., Alford, B. & Guillemin, R. *Biochem. biophys. Res. Commun.* **107**, 944–950 (1982).
15. Kobayashi, R. M., Brown, M. & Vale, W. *Brain Res.* **126**, 584–588 (1977).
16. Kewley, C. F., Millar, R. P., Berman, M. C. & Schally, A. V. *Science* **213**, 913–915 (1981).
17. Iversen, L. L. *et al. Nature* **273**, 161–163 (1978).
18. Bennett, G. W. *et al. J. Neurochem.* **32**, 1127–1130 (1979).
19. Peterfreund, R. A. & Vale, W. *Brain Res.* **239**, 463–477 (1982).
20. Srikanth, C. V. & Patel, Y. C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3930–3934 (1981).
21. Tapia-Arancibia, L., Epelbaum, J., Enjalbert, A. & Kordon, C. *Eur. J. Pharmac.* **71**, 523–525 (1981).
22. Epelbaum, J., Tapia-Arancibia, L., Kordon, C. & Enjalbert, A. *J. Neurochem.* **38**, 1515–1523 (1982).
23. Reubi, J.-C., Perrin, M., Rivier, J. & Vale, W. *Life Sci.* **28**, 2191–2198 (1981).
24. Benoit, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 917–921 (1982).
25. Benoit, R., Morrison, J., Alford, B. & Bakhtit, C. *Endocrinology* **110**, A913 (1982).
26. Weakly, J. N. *J. Physiol., Lond.* **234**, 597–612 (1973).

A hormone from the corpora cardiaca controls fat body glycogen phosphorylase during starvation in tobacco hornworm larvae

K. Siegert & R. Ziegler

Freie Universität Berlin, Institut für Tierphysiologie und Angewandte Zoologie, Grunewaldstrasse 34, 1000 Berlin 41, FRG

Steele has demonstrated the presence of a factor in the corpora cardiaca (Cc) of the adult male cockroach, *Periplaneta americana*, which increases the trehalose concentration in haemolymph¹. This factor, which was shown to be a peptide², activates fat body glycogen phosphorylase³, and glucose-1-phosphate which is liberated from glycogen is used for trehalose synthesis within the fat body. Peptides having similar properties were found in several other insects, including bees⁴, locusts⁵, stick insects^{5,6} and a lepidopteran, *Manduca sexta* (tobacco hornworm)⁷. These peptides have been called hyperglycaemic¹ or phosphorylase activating factor⁷ or trehalogon⁸. Their physiological significance during an insect's life cycle is unknown^{9,10}, and consequently it is uncertain whether these compounds are hormones or not. We have examined the activity of fat body glycogen phosphorylase during late larval development of *M. sexta*¹¹. During the moult from the fourth to the fifth instar, when the animals were unable to ingest food for a period of about 25 h, 45% of the enzyme was in an active form. Before and after the moult, only 10% of the enzyme was active. We suggested that reduced feeding activity leads to the activation of fat body glycogen phosphorylase by releasing the phosphorylase activating peptide from the Cc¹¹. We now substantiate this hypothesis in reporting results obtained with fifth instar larvae of *M. sexta* that were starved during their normally active feeding period.

Pairs of experimental animals of approximately the same weight (6–7 g) were selected after the onset of the photophase

Table 1 Activity of fat body glycogen phosphorylase in fifth instar larvae of *M. sexta* after tetrodotoxin paralysis or agar ingestion

	n	Active phosphorylase (% of total)
a		
Fed animals, untreated	7	5.1 ± 0.3
Starved animals, untreated	6	41.8 ± 4.5
Fed animals, citrate-injected	6	11.3 ± 4.3
Starved animals, citrate-injected	6	45.7 ± 4.7
TTX-paralysed animals	6	41.0 ± 5.6
b		
Diet-fed animals	5	7.3 ± 0.8
Starved animals	5	34.5 ± 2.5
Agar-fed animals	5	37.0 ± 2.0

Animals were injected with tetrodotoxin (TTX; *a*) or fed non-nutrient agar (3%; *b*). TTX (containing 5 µg citrate per 1 µg TTX; Boehringer Mannheim) was dissolved in double-distilled water (1 µg TTX µl⁻¹) and injected at 4 µg TTX per g of body weight¹⁹ using a hypodermic syringe (Hamilton) equipped with a 33-gauge needle. Control injections with citrate buffer (5 µg µl⁻¹, pH 4.8–4.9) were performed in fed and starved animals. Standard diet-fed and starved larvae were also examined. Larvae were killed 3 h after the onset of treatment. Values are mean ± s.e.m.

on day 3 of the fifth larval instar; one of each pair was starved, the other used as a fed control. At the onset of treatment, 9.2% of phosphorylase was found to be in the active form but within 3 h of starvation this had increased to 51.7%, while a value of 8.5% was observed in fed controls. After 24 h starvation phosphorylase had almost returned to the inactivated state; only 18.2% of the enzyme was in the active form (Fig. 1). The level of total haemolymph sugars did not decrease within the first 24 h of starvation (data not shown) and was presumably maintained by the increased phosphorylase activity.

Two sets of experiments were performed to define the stimulus leading to the activation of fat body glycogen phosphorylase. First, because phosphorylase could be activated as a consequence of physical activity when larvae crawl around searching for food, animals were paralysed by injecting tetrodotoxin¹². This treatment did not prevent the activation of fat body glycogen phosphorylase within 3 h of the onset of starvation (Table 1*a*). Second, the possibility that phosphorylase activation was caused by emptying of the gut was tested. When larvae were fed pure agar (3%), which is believed to be non-nutrient¹³ and is ingested like a normal diet, phosphorylase was again activated as in starved larvae (Table 1*b*). It thus seems that during starvation phosphorylase is not activated as a consequence of increased physical activity, nor simply because the gut empties.

To investigate further the role of the Cc and of the phosphorylase activating peptide, we removed the Cc (plus the corpora allata, Ca) from fifth instar larvae. Cardiacectomized animals failed to activate fat body glycogen phosphorylase within 3 h of starvation, whereas untreated and sham-operated larvae showed a normal response (Table 2*a*). Injections of extracts of larval Cc or Ca into the haemolymph of cardiacectomized—allatectomized fifth instar larvae of *M. sexta* showed that only the Cc activate glycogen phosphorylase (Table 2*b*). This result agrees with data obtained in ligated larvae⁸.

We have thus shown that glycogen phosphorylase in fat body of starved fifth instar larvae of *M. sexta* is activated by a hormone from the Cc. This activation is not caused by increased physical activity nor directly by the emptying of the gut. We conclude that the lack of ingestion of nutrients somehow induces the Cc to secrete the hormone, which is transported by haemolymph to the fat body.

A substance having glycogenolytic activity has been shown to function during flight in *Calliphora erythrocephala*¹⁴ and possibly in *Locusta migratoria*¹⁵. We have demonstrated for the first time a function for a peptide with similar properties in an insect larva and we can now call it a hormone. However, we consider it incorrect to call it hyperglycaemic or trehalocaemic,

Table 2 Fat body glycogen phosphorylase activity in cardiectomized-allatectomized larvae of *M. sexta* after 3 h of starvation, and effects of extracts of Cc or Ca

	n	Active phosphorylase (% of total)
a		
Untreated, fed animals	5	8.5 ± 0.5
Untreated, starved animals	5	33.5 ± 2.6
Sham-operated, fed animals	6	5.6 ± 0.4
Sham-operated, starved animals	6	36.6 ± 1.8
CcCa ⁺ , fed animals	5	5.2 ± 1.0
CcCa ⁺ , starved animals	5	10.9 ± 1.3
b		
CcCa ⁺ , saline-injected animals	9	9.5 ± 1.5
CcCa ⁺ , Ca-injected animals	6	8.0 ± 2.0
CcCa ⁺ , Cc-injected animals	6	58.8 ± 12.3

a, About 20 h before the starvation experiments took place, larvae were anaesthetized with carbon dioxide and the Cc and Ca removed within 10–12 min through a single incision of the stretched neck membrane. Untreated and sham-operated fed and starved specimens were examined simultaneously. Values are mean ± s.e.m. **b**, Cc and Ca were removed from the animals about 2 days before injection of extracts. Extracts were prepared as described previously⁷ using larval Cc or Ca. Lyophilized material was dissolved in insect saline containing 0.1% bovine serum albumin. One pair of organs was administered in a volume of 10 µl. The fat body was dissected 10 min after the injection of extracts. Values are mean ± s.e.m.

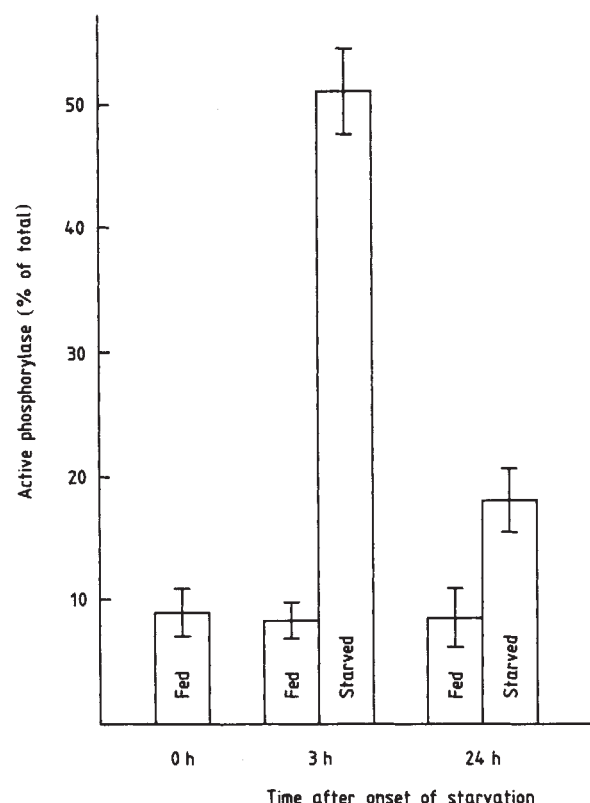


Fig. 1 Activation of fat body glycogen phosphorylase in fifth instar larvae of *M. sexta* during starvation. Larvae of *M. sexta* were reared on a standard diet²⁰. Starved and fed animals were killed after onset of treatment at the times indicated. The fat body was dissected and quickly rinsed in insect saline (110 mM KCl, 4 mM NaCl, 15 mM MgCl₂, 5 mM KH₂PO₄, 4 mM CaCl₂, pH 6.5)²¹, blotted on filter paper and homogenized in 1 ml medium (50 mM triethanolamine, 5 mM Na₂ EDTA, 20 mM NaF, 1 mM N-phenylthiourea, pH 7.0)²² with a motor-driven glass-Teflon tissue grinder. The supernatant (10 min, 13,000g) was used to determine phosphorylase activities in the direction of glycogen breakdown. Active phosphorylase was measured first in the absence and then total phosphorylase activity in the presence of 2 mM AMP (ref. 7). Values are mean ± s.e.m. from five animals tested.

or even trehalogon, for although glycogen phosphorylase is clearly activated by the peptide, only very small changes in the concentration of haemolymph sugars are observed in *M. sexta*⁷ and none in adult locusts⁴. In addition, the phosphorylase activating hormone may prove to be identical to the adipokinetic hormone of *M. sexta*¹⁶; the same has been suggested for *Locusta migratoria*^{17,18}. We therefore prefer as a preliminary name the more accurate description, 'glycogen phosphorylase activating hormone' or GPAH.

We thank Dr G. J. Goldsworthy for critical reading of the manuscript and M. Schulz, A. Reutzel, M. Rohde and T. Rohloff for care of animals. This work was supported by Deutsche Forschungsgemeinschaft (Zi 135/7-3).

Received 24 August; accepted 29 November 1982.

1. Steele, J. E. *Nature* **192**, 680–681 (1961).
2. Migliori Natalizi, G. & Frontali, N. J. *J. Insect Physiol.* **12**, 1279–1287 (1966).
3. Steele, J. E. *Gen. comp. Endocr.* **3**, 46–52 (1963).
4. Goldsworthy, G. J. *J. Insect Physiol.* **15**, 2131–2140 (1969).
5. Dutrieu, J. & Gourdoux, L. C. r. hebdom. Séanc. Acad. Sci., Paris **265**, 1067–1070 (1967).
6. Gäde, G. *Physiol. Ent.* **4**, 131–134 (1979).
7. Ziegler, R. *Gen. comp. Endocr.* **39**, 350–357 (1979).
8. Steele, J. E. in *Biology in the Future "VBW 80"* (eds Locke, M. & Smith, D. S.) 369–383 (Academic, New York, 1980).
9. Highnam, K. C. & Hill, L. *The Comparative Endocrinology of the Invertebrates* (Arnold, London, 1977).
10. Steele, J. E. *Adv. Insect Physiol.* **12**, 239–323 (1976).
11. Ziegler, R., Siebert, K. & Rohde, M. *Verh. Dtsch. Zool. Ges.*, 303 (1978).
12. Williams, C. M. *Science* **160**, 444 (1968).
13. Bhaskaran, G. & Jones, G. J. *J. Insect Physiol.* **26**, 431–440 (1980).
14. Vejbjerg, K. & Normann, T. C. J. *J. Insect Physiol.* **20**, 1189–1192 (1974).
15. Van Marrewijk, W. J. A., Van Den Broek, A. Th. M. & Beenackers, A. M. Th. *Insect Biochem.* **10**, 675–679 (1980).
16. Ziegler, R. & Schulz, M. *Acta endocr. Suppl.* **246**, 32–33 (1982).
17. Holwerda, D. A., Van Doorn, J. & Beenackers, A. M. Th. *Insect Biochem.* **7**, 151–157 (1977).
18. Goldsworthy, G. J., Mordue, W. & Guthkelch, J. *Gen. comp. Endocr.* **18**, 545–551 (1972).
19. Nijhout, H. F. J. *J. Insect Physiol.* **22**, 453–463 (1976).
20. Truman, J. W. J. *exp. Biol.* **57**, 805–820 (1972).
21. Jungreis, A. M., Jatlow, P. & Wyatt, G. R. J. *J. Insect Physiol.* **19**, 225–233 (1973).
22. Ziegler, R. et al. *J. comp. Physiol.* **131**, 321–332 (1979).

A severe combined immunodeficiency mutation in the mouse

Gayle C. Bosma, R. Philip Custer & Melvin J. Bosma

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA

The most debilitating human lymphoid deficiency disease, known as severe combined immunodeficiency (SCID), impairs the differentiation of both T and B lymphocytes^{1–7}. Affected infants are highly susceptible to recurring infections of viruses, fungi and bacteria and invariably die within 2 yr of birth. Inheritance of this congenital syndrome may show X-linked^{8,9} or autosomal recessive control^{1,2,9}. To date autosomal recessive inheritance of SCID has been observed in Arabian foals¹⁰ which represent the only known animal model of this disease syndrome but here we report an autosomal recessive mutation in mice that severely impairs lymphopoiesis. Mice homozygous for this mutation have few if any lymphocytes; consequently they are hypogammaglobulinaemic and deficient for immune functions mediated by T and B lymphocytes. These mice, therefore, represent a new model for investigating how lymphoid differentiation may be impaired in the disease state and regulated in the normal state.

In the course of radioimmune quantitation of serum immunoglobulin (Ig) isotypes in specific pathogen-free (SPF) mice, we noted four littermates of the C.B-17 inbred strain [BALB/c.C57BL/Ka-Igh-1^b/ICR (N17F34)] that had no detectable IgM, IgG1 or IgG2a (≤1 µg ml⁻¹); immunodiffusion assays failed to detect IgG3, IgG2b and IgA. Subsequent analysis of the pedigree of these mice (Fig. 1) showed their defect to be heritable and under the control of a recessive gene (*scid*). Two of the defective progeny were found to contain a single

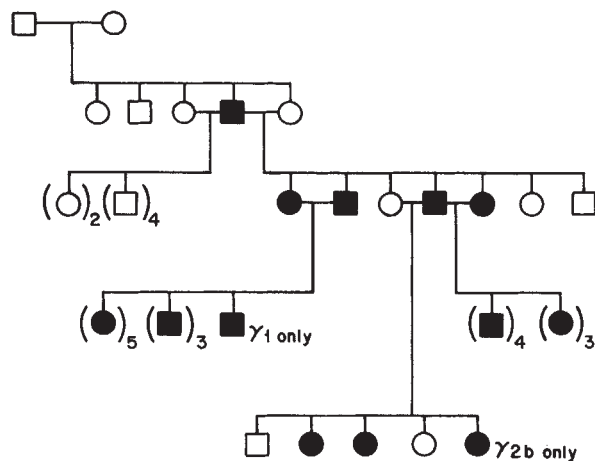


Fig. 1 Partial pedigree of C.B-17scid ancestor mice showing recessive inheritance of SCID syndrome. Females (●) and males (■) denote the absence of detectable ($\leq 1 \mu\text{g ml}^{-1}$) serum immunoglobulin (IgM, IgG3, IgG1, IgG2, IgG2a and IgA). The numbers in subscript indicate number of siblings; $\gamma 1$ only and $\gamma 2b$ only refer to two mice that lacked all of the above isotypes except IgG1 and IgG2b, respectively. Assays for Ig isotypes included immunodiffusion in micro-Ouchterlony plates and radio-immune assay and used class-specific antisera (Litton Bionectics) together with appropriate myeloma proteins as internal standards²⁰.

Table 1 Percentages of pre-B, B and Thy-1⁺ cells in lymphoid tissues of individual mice

Mouse	Bone marrow pre-B cells (2C2 ⁺)	Spleen cells (lyb 8.2 ⁺)	(Ig ⁺)	(thy-1 ⁺)	Thymus (thy-1 ⁺)
C.B-17 (+/+)		51	55	46	96
C.B-17		42	37	32	94
C.B-17scid/+		43	43	33	98
C.B-17scid/+	21	37	41		
C.B-17scid/+	36	35	53		
C.B-17scid	≤ 1	4	≤ 1		
C.B-17scid	2	≤ 1	≤ 1	≤ 3	
C.B-17scid	≤ 1	2	≤ 1	12	
C.B-17scid	≤ 1	≤ 1	2	13	
C.B-17scid		8	7	27	70
C.B-17scid		≤ 1	≤ 1	11	35
C.B-17scid			4	25	82
C.B-17scid			≤ 1	15	84
C.B-17scid			≤ 1	13	81
C.B-17scid			≤ 1	11	80
C.B-17scid			≤ 1	5	87
C.B-17scid			≤ 1	10	89

Dispersed cell populations ($\sim 10^6$) from bone marrow, spleen and thymus were incubated with various antibody preparations for 45 min at 4 °C in 0.1 ml of Hanks' balanced salt solution (without phenol red) supplemented with 0.1% bovine serum albumin and 0.1% NaN₃. The antibody preparations included mouse monoclonal antibody (IgG1) specific for a B-cell determinant (Lyb-8.2)¹³; rat monoclonal antibody specific for a pre-B cell determinant (2C2)¹⁴; fluorescein isothiocyanate (FITC)-conjugated rabbit anti-immunoglobulin specific for heavy and light immunoglobulin chains; and rat monoclonal antibody (30-H12)¹⁹ specific for Thy-1.2. FITC-conjugated heterologous antisera to rat immunoglobulin (Miles) to mouse IgG1 (provided by Dr Subbarao), and to mouse immunoglobulin (Fab)₂ (Cappel) were used as second-stage reagents where appropriate. Cells were washed twice after each incubation, counter stained with 10 μl of ethidium bromide (100 $\mu\text{g ml}^{-1}$) for 10 min at room temperature and analysed on a FACS II (Becton Dickinson). Cells stained with ethidium bromide (dead cells) were excluded from analysis.

immunoglobulin isotype; one produced IgG1 only, another IgG2b only. We have since established a colony of SPF mice homozygous for the defective gene (C.B-17scid). By testing over 600 progeny of various test crosses we have shown scid segregates as a single autosomal recessive gene and that it is not linked to the immunoglobulin heavy chain (Igh) locus or the major histocompatibility (H-2) locus; further, scid does not cause a deficiency of adenosine deaminase, an enzyme often absent in patients that show autosomal recessive inheritance of their SCID syndrome^{11,12} (G.C.B. & M.J.B., unpublished data).

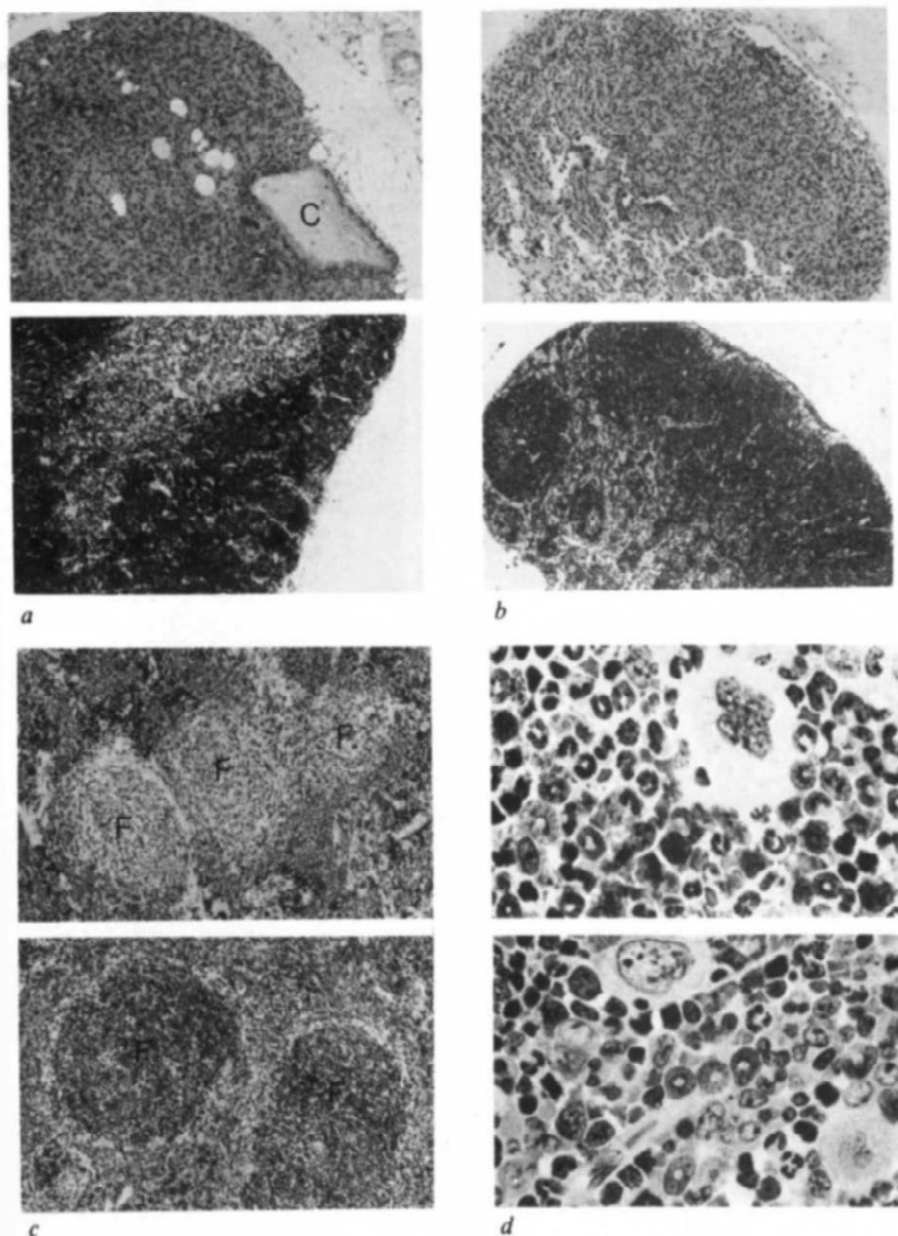
C.B-17scid mice are deficient for both B and T lymphocyte function. In addition to being hypogammaglobulinaemic, C.B-17scid mice are unable to produce specific antibody against either of two T-independent antigens (that is, bacterial levan and trinitrophenyl-Ficoll). Moreover, spleen cells of C.B-17scid mice do not proliferate in response to lipopolysaccharide, a B-cell mitogen. Similarly, with respect to T-cell function, C.B-17scid splenic cells do not proliferate in response to the T-cell mitogen, concanavalin A, nor to allogeneic lymphocytes in a one-way mixed lymphocyte reaction. The most dramatic indication of T-cell deficiency is the inability of C.B-17scid mice to reject skin allografts. Unlike C.B-17 control mice which reject full thickness skin grafts of C57BL/6 or (B6.C-9 $\times$ C57BL/6)F₁ mice within 3 weeks of grafting, C.B-17scid recipients allow such grafts to heal and grow hair. Three C.B-17scid mice grafted with C57BL/6 and eight with (B6.C-9 $\times$ BALB/c)F₁ skin all retained their grafts (≥ 200 days).

C.B-17scid mice are leukopenic. Their lymphoid organs are 1/10th or less normal size when uncomplicated by infection, anaemia or neoplasia; Peyer's patches of the intestinal tract are rarely visible. In Fig. 2, histological sections are contrasted with comparable tissues from C.B-17scid/+ heterozygotes. No lymphocytic cortex is evident in C.B-17scid thymus, splenic follicles are virtually devoid of lymphocytic cells, and lymph nodes have only a sparse scattering of lymphoid cells. All of these sites consist mostly of vascularized supportive tissue with variable numbers of fibroblasts, histiocytes and occasional plasmacytes; macrophages are frequently conspicuous in lymph nodal sinuses. Epithelial components of the thymus are invariably present, often found as proliferating tufts. The bone marrow of C.B-17scid and its heterozygous counterpart are within the norm, reacting as usual to incidental infection or anaemia with granulocytic or erythroid hyperplasia, sometimes associated with marked megakaryocytosis. Likewise, the interfollicular region (red pulp) of the spleen reacts under the same circumstances, indicating that myeloid differentiation is not affected by scid.

C.B-17scid mice lack detectable B cells (Table 1). Neither of two B-cell markers, Ig and Lyb-8.2 (ref. 13), can be detected in the spleen, even though 30–50% of splenic cells from C.B-17scid/+ heterozygotes bear these surface markers. Moreover, we have not been able to detect pre-B cells in C.B-17scid mice. Monoclonal antibody reactive with a surface marker on pre-B cells¹⁴ fails to stain bone marrow cells of C.B-17scid mice (Table 1). In agreement, fluoresceinated antisera specific for IgM heavy chains (μ) fail to detect cytoplasmic μ in C.B-17scid bone marrow cells (data not shown).

Most C.B-17scid mice, despite their small thymus (1/10th to 1/100th normal size) and lack of functional T cells, show the Thy-1 marker on a majority of recovered cells ($0.3\text{--}0.7 \times 10^6$ per thymus) from the thymus (Table 1). These cells are considerably larger than normal thymocytes and express more Thy-1 antigen. Although Thy-1 is not exclusively a T-cell marker^{15,16} many of the C.B-17scid Thy-1⁺ cells may be immature T cells as we have found spontaneous T-cell lymphomas in C.B-17scid mice. These tumours have been detected in $\geq 10\%$ of C.B-17scid mice 5–9 months of age; they appear to arise in the thymus, are highly invasive and transplantable. The pathology of an affected lymph node is shown in Fig. 3. Eight of 9 tumours have been serotyped; all display Thy-1 but lack immunoglobulin determinants. Three tumours examined all express the specific T-cell markers Lyt-1 and Lyt-2.

Fig. 2 Histology of C.B-17^{scid} mice (upper panels) compared with (C.B-17^{scid}/+ heterozygotes (lower panels). Tissues were fixed in Carson's buffered neutral formol, embedded in glycol methacrylate (Sovall), sectioned at 2 μ m, and stained with hematoxylin and eosin. *a*, Thymus; the C.B-17^{scid} gland is completely devoid of the dense lymphocytic cortex found in the heterozygote, resembling the medulla of the latter. It is made up of vascularized fibrous supportive tissue containing the epithelial components and a relatively sparse scattering of histiocytes and lymphoid cells. Mucinous cysts (C) similar to those found in thymic sites of nude athymic mice (*nu/nu*) are occasionally encountered. *b*, Lymph node; the framework of the C.B-17^{scid} node is poorly populated with lymphocytes; marginal and hilar sinuses are usually well formed and contain macrophages. The node of the heterozygote is rich in lymphocytes, especially dense in the cortex. *c*, Spleen; the follicles (f) of C.B-17^{scid} spleens are devoid of lymphocytes, their cellular population consisting chiefly of fibroblasts and histiocytes, in sharp contrast with the normal lymphoid follicles seen in the heterozygote. In each instance, the haematopoietic components of the interfollicular red pulp (erythroid, granulocytic and megakaryocytic) may proliferate extensively provided the appropriate stimulus, such as anaemia or infection, obtains and the organ may enlarge greatly. *d*, Bone marrow; no significant differences are found between the marrow of uncomplicated C.B-17^{scid} mice and heterozygotes, although infection can bring about conversion to a virtually total granulocytic state with marked immaturity. *a-c*, $\times 80$; *d*, $\times 650$.



To see whether C.B-17^{scid} mice have a defective cellular environment for lymphoid differentiation, we made reciprocal cell transfers between C.B-17^{scid} and BALB/c mice. These strains share the same genetic background including the same *H-2* complex but differ at the *Igh* locus. By use of the *Igh* allotypes as a marker we were able to test whether C.B-17^{scid} mice would support B-cell differentiation of BALB/c bone marrow cells and vice versa. We were also interested in testing the differentiative potential of C.B-17^{scid} bone marrow and fetal liver cells in lethally X-ray irradiated (B6.C-9 $\times$ BALB/c) F_1 recipients (B6.C-9 mice have the *Igh* allotype of BALB/c but otherwise are not known to differ from the C57BL/6 strain). These F_1 recipients enabled us to use both *Igh* and *H-2* markers in examining donor and self components in the repopulated mouse.

The results of the cell transfer experiments are shown in Table 2. All C.B-17^{scid} recipients of BALB/c bone marrow produced only immunoglobulins of BALB/c allotype 4 weeks after cell transfer. In contrast, none of the BALB/c recipients of C.B-17^{scid} bone marrow or fetal liver cells showed the C.B-17 allotype, including the (B6.C-9 $\times$ BALB/c) F_1

recipients, but they were rescued from the lethal effects of X-ray irradiation. The reason for the continued production of immunoglobulins of host allotype in F_1 recipients is unclear but may reflect secretion by long-lived plasma cells (J. Sprent, personal communication). Interestingly, the erythrocytes of F_1 recipients in groups *f* and *g* were entirely of the C.B-17^{scid} haplotype (*H-2d*). But erythrocytes are derivatives of myeloid differentiation, which our histological evidence indicates is not defective in C.B-17^{scid} mice.

We conclude C.B-17^{scid} mice are severely deficient in immunoglobulin stem cells even though their cellular environment readily supports the differentiation of such cells. In fact, some C.B-17^{scid} mice produce detectable levels of one or more immunoglobulin isotype. So far we have tested a total of 206 C.B-17^{scid} mice for immunoglobulin heavy and light chain isotypes. Thirty-one of these had low levels of serum immunoglobulin; 14 mice showed a single IgG isotype (IgG1, IgG2b, or IgG3) and 17 showed IgM and two or more IgG isotypes. We have yet to detect IgG2a or IgA in C.B-17^{scid} mice, even though there is no apparent defect in the structural genes for these two classes, that is, (BALB/c $\times$ C.B-17^{scid}) F_1 mice do

Table 2 Transfer of bone marrow or fetal liver cells between C.B-17^{scid} and normal mice; analysis of allotype and H-2 markers in recipients

Group	Donor	Cells	Recipient	Donor allotype	No. recipients positive/No. tested		
					Recipient allotype	Donor H-2	Recipient H-2
a	BALB/c	BM(3×10^6)	C.B-17 ^{scid}	4/4	0/4		
b	BALB/c	BM(1.5×10^6)	C.B-17 ^{scid}	3/3	0/3		
c	C.B-17	BM(3×10^6)	BALB/c	5/5	5/5		
d	C.B-17 ^{scid}	BM(3×10^6)	BALB/c	0/4	4/4		
e	C57BL/6	BM(8×10^6)	(B6.C-9 ×BALB/c)F ₁	7/7	7/7	7/7	0/7
f	C.B-17 ^{scid}	BM(8×10^6)	(B6.C9 ×BALB/c)F ₁	0/5	5/5	5/5	0/5
g	C.B-17 ^{scid}	BM(8×10^6)	(B6.C-9 ×BALB/c)F ₁	0/10	10/10	10/10	0/10
h	C.B-17	FL(7×10^6)	(B6.C-9 ×BALB/c)F ₁	9/9	9/9		
i	C.B-17 ^{scid}	FL(7×10^6)	(B6.C-9 ×BALB/c)F ₁	0/7	7/7		

Bone marrow cells (BM) were taken from 12–20 week-old donors and injected intravenously in 0.3 ml of Hanks' balanced salt solution containing 1% fetal calf serum. The number of cells injected is indicated in parentheses. Before injection, the cells in experiments e and f were treated with anti-Thy-1.2 plus complement to remove contaminating Thy-1⁺ cells as a possible variable. Fetal liver (FL) cells of 17–18 day fetuses were used in groups h and i. One day before injection with donor cells, BALB/c and (B6.C-9×BALB/c)F₁ recipients were X-ray irradiated with 550 rad and 950 rad, respectively. The conditions were as follows: 225 kV; 15 mA; filtration 1 mm Al and 0.5 mm Cu; dose rate 40–45 rad min⁻¹. The allotype results cover a 4–15-week interval after cell transfer. At 4, 6, 9, 12 and 15 weeks after injection, recipients were bled and their sera tested in micro-Ouchterlony for the presence of BALB/c Igh allotypes (Igh-1a, Igh-4a) and for C57Bl and C.B-17 Igh allotypes (Igh-1b, Igh-4b). The antisera used have been described previously²⁰. H-2 typing of mouse erythrocytes was done at 6 and 12 weeks after cell transfer using a modified version²¹ of the polyvinylpyrrolidone haemagglutination method²². Antisera specific for the H-2^d (C3H.SW/Hz anti-BALB/c) and for the H-2^b [(CBA/N×BALB/c)F₁ anti-C57BL/6] were supplied by Dr R. Riblet and K. Stabenow.

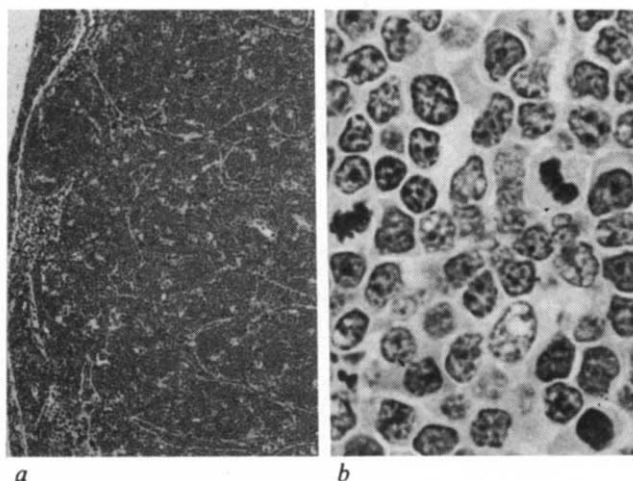


Fig. 3 Section of lymph node of C.B-17^{scid} mouse with spontaneous T-cell lymphoma. Such tumours characteristically grow in diffuse sheets (a) of poorly differentiated and rapidly growing lymphoid cells (b), are transplantable to C.B-17 mice, and adopt a consistent growth pattern both in spontaneous and transplanted states. a, $\times 80$; b, $\times 250$.

produce IgG2a and IgA of the C.B-17 allotype (unpublished data).

Our detection of immunoglobulin and Thy-1⁺ cells in C.B-17^{scid} mice indicates B and T lymphoid differentiation may occur at a very low rate. Interestingly, of the few T and B cells to arise, only the T cells seem highly disposed to neoplasia; B-cell tumours have not been observed. Although the basis for spontaneous T cell lymphomas in C.B-17^{scid} mice remains to be elucidated, their appearance is relevant to reports^{17,18} of increased lymphoid malignancy in patients with immune deficiency diseases.

The ability of most C.B-17^{scid} mice to survive ≥ 1 yr is puzzling. They have been maintained in a clean environment as SPF mice which in part may be responsible, because we have not been very successful in raising C.B-17^{scid} mice in a conven-

tional animal facility where most of these mice have succumbed to infections of mouse hepatitis virus and/or *Pasteurella pneumotropica*. However, the SPF environment is not germ-free and one might question to what extent a few lymphocytes together with various host protective mechanisms (macrophages, natural killer cells) may compensate for the *scid* defect. Nevertheless, the importance of the C.B-17^{scid} mouse is clear both as a model for understanding lymphocyte differentiation and regulation and for understanding the relationship between immune deficiency and spontaneous T cell lymphomas.

We thank R. L. Coffman, J. Ledbetter, B. Subbarao, F. W. Symington, and R. Riblet for antibodies; R. W. Overton, K. Stabenow, T. Lerro, G. Marshall, E. Cunningham, C. Congleton and G. Kroetz for help and J. Mull for typing this manuscript. This work was supported by NIH grants A1 13323, CA 04946 and CA 06927, by funds from the Commonwealth of Pennsylvania and from the W. W. Smith Charitable Foundation.

Received 21 September; accepted 24 November 1982.

- Glanzman, E. & Riniker, P. *Ann. Paediat. (Basel)* **175**, 1–32 (1950).
- Tobler, R. & Cottier, H. *Helv. paediat. Acta* **13**, 313–338 (1958).
- Hitzig, W. H., Biro, Z., Bosch, H. & Huser, H. J. *Helv. paediat. Acta* **13**, 551–585 (1958).
- Breton, A., Walbaum, R., Boniface, L., Goudemand, M. & Dupont, A. *Arch. Franc. Pediat.* **20**, 131–146 (1963).
- Nezelof, C., Jammet, M. C., Lortholary, P., Labrune, B. & Lamy, M. *Arch. Franc. Pediat.* **21**, 897–920 (1964).
- Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. I. & Meuwissen, H. J. *Lancet* **ii**, 1067–1069 (1972).
- Wild Hth Org. Rep. *Clin. Immun. Immunopath.* **2**, 415–445 (1974); **13**, 296–359 (1979).
- Gitlin, D., Janeway, C. A., Apt, L. & Craig, J. M. in *Cellular Humoral Aspects of Hypersensitive States* (ed. Lawrence, H. S.) 375–441 (Hoeber, New York, 1959).
- Hoyer, J. R., Cooper, M. D., Gabrielson, A. E. & Good, R. A. *Medicine* **47**, 201–226 (1968).
- McGuire, T. C., Banks, K. L. & Poppie, M. J. *Clin. Immun. Immunopath.* **3**, 555–566 (1975).
- Ackeret, C., Plüss, H. J. & Hitzig, W. G. *Pediat. Res.* **10**, 67–70 (1976).
- Hirschhorn, R. & Martin, D. W. *Springer Semin. Immunopath.* **1**, 299–321 (1978).
- Symington, F. W., Subbarao, B., Mosier, D. E. & Sprent, J. *Immunogenetics* (in the press).
- Coffman, R. L. & Weissman, I. L. *J. exp. Med.* **153**, 269–279 (1981).
- Schrader, J. W., Batlye, F. & Scollay, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4161–4165 (1982).
- Basch, R. S. & Berman, J. W. *Eur. J. Immun.* **12**, 359–364 (1982).
- Gatti, R. A. & Good, R. A. *Cancer* **28**, 89–89 (1971).
- Kersey, J. H., Spector, B. D. & Good, R. A. *Int. J. Cancer* **12**, 333–347 (1973).
- Ledbetter, J. A. & Herzenberg, L. A. *Immun. Rev.* **47**, 63–90 (1979).
- Bosma, G. C. et al. *J. Immun.* **124**, 879–884 (1990).
- Watson, J. & Riblet, R. *J. exp. Med.* **140**, 1147–1161 (1974).
- Rubinstein, P. & Kaliss, N. *Transplantation* **17**, 121 (1974).

HLA-DR products are a subset of human Ia antigens

M. W. Makgoba, S. V. Fuggle, A. J. McMichael* & P. J. Morris

Nuffield Department of Surgery and * Nuffield Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK

Human Ia antigens are polymorphic cell-surface sialoglycoproteins which have restricted tissue distribution^{1,2}. They are bimolecular complexes of 34,000 (α) and 28,000 (β) molecular weight and most of the polymorphism is found in the smaller polypeptides³⁻⁵. They are involved in the initiation of immune responses⁶ and particular Ia antigens are associated with increased susceptibility to certain diseases⁷. They are also the major barrier to human allogeneic tissue transplantation⁸. Whereas serological analysis and mixed lymphocyte typing have defined three polymorphic families of Ia antigens, HLA-DR, -DC and -SB⁹⁻¹⁷, protein sequencing results¹⁸ and studies with monoclonal antibodies indicate that the complexity is much greater¹⁹⁻²³. Thus the HLA-DR and DC specificities as defined by alloantisera, could represent groups of antigens which are controlled by HLA genes in linkage disequilibrium. Here, we have used a monoclonal antibody specific for HLA-DR2 to show that this determinant is carried by molecules which are distinct from those of the DC series and which represent 30% of the Ia antigens expressed on the cell surface of an HLA homozygous line PGF.

NDS-1538 has been shown in a separate study to bind only to HLA-DR2 positive cells²⁴. There is no cross reactivity with HLA-DR1 or -DR6, so that it is not recognizing the DC1 (MT1, MB1, LB12) specificity seen by other monoclonal anti-

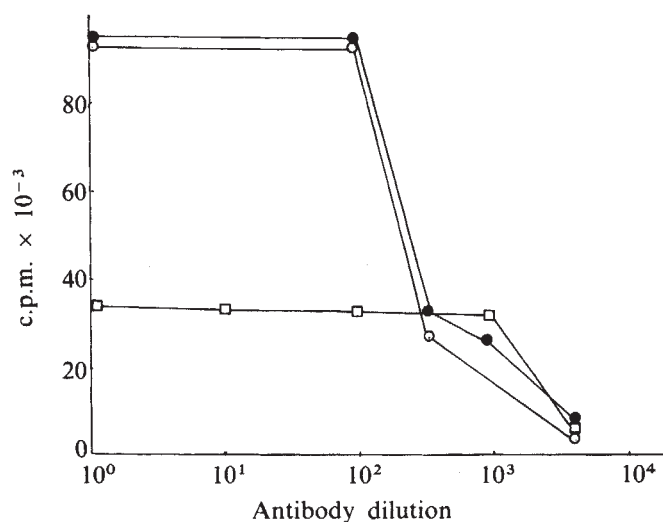


Fig. 1 Saturating binding assay. PGF cells grown in RPMI 1640 (Gibco) were washed three times in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 100 μ l (5×10^5 cells) per assay tube were used. DEAE-Sephacrose (Pharmacia) column purified ascites of antibodies NFK 2 and NDS-1538 were added neat, 1:10, 1:100, 1:500, 1:1,000 and 1:5,000 dilutions. 30 μ l of antibody was added per assay tube and incubated for 90 min at 4 °C. Excess unbound antibody was removed by washing three times in 0.1% BSA-PBS. ¹²⁵I-labelled rabbit F(ab)₂ anti-mouse F(ab)₂ (¹²⁵I-RAM) was diluted to 10 μ Ci ml⁻¹ and 25 μ g ml⁻¹ in 0.5% BSA-PBS; 25 μ l was added per assay tube and incubated for 90 min. Excess unbound ¹²⁵I-RAM was removed by three washes in 0.5% BSA-PBS and the assay tubes were counted in a gamma counter. Results are shown as bound counts per minute (c.p.m.) for NFK2 (○), NDS-1538 (□) and NFK2 + NDS-1538 (●).

bodies²⁵⁻²⁷. Table 1 summarizes the typing results on lymphoblastoid cell lines prepared from caucasian blood donors. A perfect correlation was found with HLA-DR2 defined by alloantisera. One discrepancy observed was an oriental individual typed as HLA-DR2 by alloantisera but whose cells did not react with NDS-1538.

NFK2 is a monoclonal antibody that is specific for a monomorphic determinant that appears to be found on all human Ia antigens as it has been shown to react with antigens of the DR, DC and SB series on mutant cells lines (S. Shaw, personal communication). To determine the amount of HLA-DR2 defined by NDS-1538 relative to the total Ia antigen recognized by NFK2, saturating binding assays were carried out using these antibodies on the HLA-DR2 consanguineous homozygous cell line PGF. The results (Fig. 1) show that the NDS-1538 detected 30% of the total Ia on the cell surface.

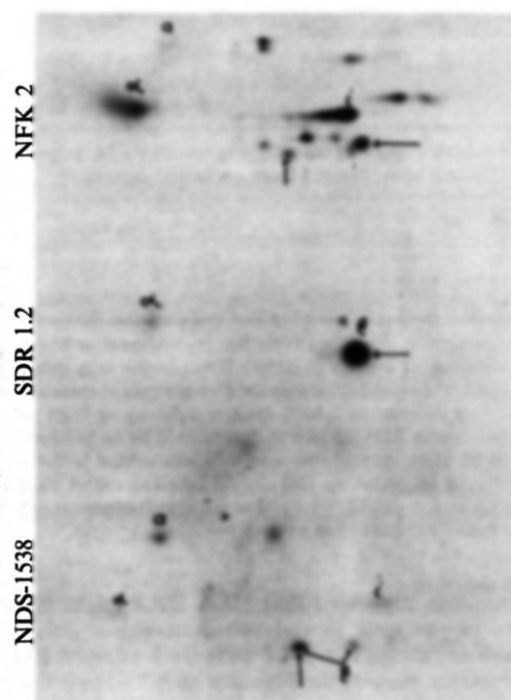


Fig. 2 Two-dimensional gel electrophoresis. 5×10^7 PGF cells grown in RPMI-1640 were washed three times in PBS and labelled metabolically with 0.5 mCi ³⁵S-methionine (Amersham) for 6 h at 37 °C in RPMI methionine-free medium (Gibco). The reaction was stopped with cold 4 °C PBS. The cells were lysed with 1.5 ml PBS containing 0.5% NP40 (Sigma), 1 mM phenylmethylsulphonyl fluoride (Sigma) and 1 IU ml⁻¹ Aprotinin (Sigma). The nuclei were pelleted at 3,000g for 30 min and the lysate passed over a 2-ml lentil lectin Sepharose column (Pharmacia) at 4 °C. The column was washed with 40 ml of 0.5% NP40 PBS and was eluted with 10% α -methylmannoside (Sigma). Immunoprecipitates were made by adding 5 μ l of antibody NFK 2, SDR1.2 or NDS-1538 to 200 μ l of labelled glycoprotein. After 1 h of incubation, 10 μ g of rabbit anti-mouse immunoglobulin (Miles) was added for 1 h. The complexes were centrifuged and washed three times with 0.5% NP40 PBS and eluted with 9.2 M urea (Becton Dickinson) buffer. The samples (30 μ l) were loaded onto the acid end of 120 mm $\times$ 2 mm diameter tube gels made of 5.5 g urea, 1.33 ml of 30% acrylamide (Biorad), 2 ml of 10% NP40, 1.97 ml of H₂O, 0.5 ml of pH 3.5-10 ampholines (Biorad). They were run at 750 V for 4 h and then placed on top of a SDS (Biorad) slab gel run at 30 mA for 4 h. The gels were fixed and dried and exposed to a Kodak X-OMAT S film for 14 days at -70 °C. They were developed and the patterns compared. The results for precipitates made with NFK2, SDR1.2 and NDS-1538 are shown. a indicates the α chains; i marks the invariant spot; β indicates the cluster of β chains. Spots that correspond exactly are marked with arrows $\uparrow$ and $\leftarrow$.

Fig. 3 Competitive inhibition experiment. In *a*, ^{125}I -NFK 2 is tested against PA2.6 (monoclonal anti-HLA ABC) (○). NDS-1538 (●) and cold NFK 2 (■). In *b* ^{125}I -NDS-1538 is tested against PA2.6 (○), NFK 2 (■) and cold NDS-1538 (●). Serial dilutions from 1 in 10 ascitic fluid, of the unlabelled antibodies to be used as inhibitors were dispensed in 25 μl aliquots into 24 tubes. 50 μl of buffer containing 2.5×10^5 viable PGF cells were added to each tube. After 1 h 25 μl of ^{125}I -NFK 2 ($0.2 \mu\text{g ml}^{-1}$) or ^{125}I -NDS-1538 ($0.2 \mu\text{g ml}^{-1}$) was then added to each tube. After a further incubation at 4°C for 1 h, the cells were washed three times to remove excess unbound antibody, resuspended in fresh buffer, transferred to clean tubes and counted for radioactivity.

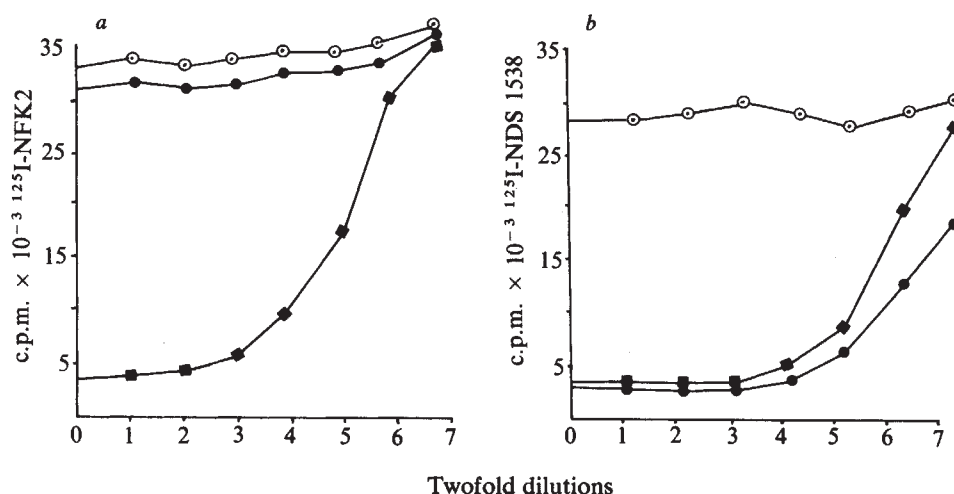


Table 1 2×2 table showing tissue typing results on 34 lymphoblastoid cell lines, derived from caucasian donors

		DR2 by alloantisera	
		+	-
DR2 by NDS-1538	+	10	0
	-	0	24

The comparison is made between the monoclonal antibody NDS-1538 and alloantisera known to define DR2. Seven sera monospecific for HLA-DR2 were used, in a standard complement-dependent lysis assay, and + is defined as $>60\%$ lysis of cells by at least six of the sera. Reactivity with NDS-1538 was determined by an indirect binding assay where 50 μl of monoclonal antibody was added to 5×10^5 lymphoblastoid cells followed by 100 μl ^{125}I -labelled rabbit F(ab) $_2$ anti-mouse F(ab) $_2$ at $0.1 \mu\text{g ml}^{-1}$. (+) Was assigned when counts bound were more than five times background.

These findings suggested that HLA-DR2 might be a sub-population of the total Ia and two-dimensional gel analyses were therefore carried out. PGF cells were labelled internally with ^{35}S -methionine and then lysed with NP40 and the nuclei pelleted. The lysate was passed over a lentil lectin column, eluted with α -methylmannoside and immunoprecipitates made from the glycoprotein fraction using NDS-1538 and NFK 2. In addition a monoclonal antibody SDR1.2 which recognizes the DR1,2,6 associated specificity DC1 was used²⁶. Immunoprecipitates were first run under non-equilibrium pH gradient electrophoresis and these gels loaded onto SDS-polyacrylamide electrophoresis gels according to the method of O'Farrell²⁸. The two-dimensional autoradiographic patterns of NFK2, NDS-1538 and SDR1.2 showed that while NFK 2 detected several Ia antigen peptides in the β -chain region, SDR1.2, as has been described before²⁶, and NDS-1538 detected only some of the total NFK 2 spots (Fig. 2). To confirm that the peptides precipitated by NDS-1538 were part of those precipitated by NFK 2, a competitive inhibition experiment was carried out. PGF cells were incubated with serial twofold dilutions of either unlabelled NDS-1538 or NFK 2 for 1 h at 4°C , then either ^{125}I -NDS-1538 or ^{125}I -NFK 2 were added. Incubation was continued for a further hour at 4°C . After washing, the cells were counted. The results, shown in Fig. 3, indicate that NFK 2 completely inhibits binding of NDS-1538 although the reverse was not true. This result is consistent with the DR2 antigen being included in those seen by NFK 2. Sequential depletion experiments through NFK 2 and NDS-1538 immunoabsorbent columns have also confirmed this finding (data not shown).

The results indicate therefore that HLA-DR2 as defined by monoclonal antibody NDS-1538 comprises only 30% of the

total Ia antigens on the HLA-DR2 homozygous cell line. Analysis of the β chains by two-dimensional gels showed that monoclonal anti-HLA-DR2 precipitated two of the five peptide spots, seen by antibody specific for a monomorphic determinant on all Ia antigens. No such distinction could be made in the α chain, however. These findings are compatible with the hypothesis that the DR2 β chain is the product of a single locus within the human *D* region, if the division into two spots is due to post-synthetic modification. Corresponding analysis of the DC1 antigen β chain, confirming previous observations of de Kretser *et al.*²⁶, suggest that this is also the product of a distinct locus.

HLA-DR and HLA-DC antigens do not add up to the total human Ia antigens. It remains to be determined whether the remaining spots seen in the β region on our gels are the product of the *SB* locus defined by Shaw *et al.*¹⁶ Our findings suggest that the term DR should be defined to the products of a single locus within the *HLA-D* region.

We thank Drs J. E. K. Hildreth, M. J. Crumpton and A. Ting for discussion and gifts of monoclonal antibodies. This work was supported by grants from the MRC South Africa and Nuffield Dominion Trust to M. W. M. and from the MRC to A. J. McM. and P. J. M.

Received 1 October; accepted 6 December 1982.

1. Bodmer, W. F. (ed.) *Br. med. Bull.* **34**, 213-316 (1978).
2. Hart, D. J. *et al. Transplantation* **31**, 428-433 (1981).
3. Silver, J. & Ferrone, S. *Nature* **279**, 436-437 (1979).
4. Shackelford, D. A. & Strominger, J. L. *J. exp. Med.* **151**, 144-165 (1980).
5. Charron, D. J. & McDevitt, H. O. *J. exp. Med.* **152**, 188-366 (1980).
6. Munro, A. & Waldmann, H. *Br. med. Bull.* **34**, 253-259 (1978).
7. Dausset, J. & Sveigaard, A. *HLA and Disease* (Munksgaard, Copenhagen, 1977).
8. Morris, P. J., Batchelor, J. R. & Festenstein, H. *Br. med. Bull.* **34**, 259-262 (1978).
9. Mann, D. L., Abelson, L., Harris, S. & Amos, D. B. *Nature* **259**, 145-146 (1977).
10. Park, M. S., Terasaki, P. I., Bernoco, D. & Iwaki, Y. *Transplant Proc.* **10**, 823-828 (1976).
11. Tsuji, K. *et al. Transplant Proc.* **11**, 1792-1795 (1976).
12. Suciu-Foca, N. *et al. Transplant Proc.* **11**, 1781-1787 (1976).
13. Katagiri, M. *et al. Immunogenetics* **9**, 335-351 (1979).
14. Tanigaki, N., Tosi, R., Pressman, D. & Ferrara, G. B. *Immunogenetics* **10**, 151-167 (1980).
15. Markert, L. M. & Cresswell, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6101-6104 (1980).
16. Shaw, S., Kavathas, P., Pollack, M. S., Charnot, D. & Mawas, C. *Nature* **293**, 745-747 (1981).
17. McMichael, A. J. & Makgoba, M. W. *Nature* **293**, 701-702 (1981).
18. Kratzin, H. *et al. Z. physiol. Chem.* **362**, s1665 (1981).
19. Lampson, L. A. & Levy, R. J. *Immun.* **125**, 292-299 (1980).
20. Nadler, L. M. *et al. Nature* **290**, 591-593 (1981).
21. de Kretser, T. A., Crumpton, M. J., Bodmer, J. G. & Bodmer, W. F. *Eur. J. Immun.* **12**, 214-221 (1982).
22. Accolla, R. S., Gross, N., Carrel, S. & Corte, G. *Proc. 14th Int. Leucocyte Conference* (eds Resch, K. & Kirchner, H.) 195-197 (Elsevier/North Holland, Amsterdam, 1981).
23. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. *Immun. Rev.* **47**, 3-61 (1979).
24. Fuggle, S., Kirkley, J., Ting, A. & Morris, P. J. *Eur. J. Immun.* (submitted).
25. Corte, G. *et al. Nature* **292**, 357-360 (1981).
26. de Kretser, T. A., Crumpton, M. J., Bodmer, J. G. & Bodmer, W. F. *Eur. J. Immun.* **12**, 600-606 (1982).
27. Shackelford, D. A., Mann, D. L. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4566-4570 (1981).
28. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. *Cell* **12**, 1133-1142 (1977).

Abnormal mRNA for argininosuccinate synthetase in citrullinaemia

Tsung-Sheng Su, Arthur L. Beaudet
& William E. O'Brien

Departments of Pediatrics and Cell Biology, Baylor College of Medicine, Houston, Texas 77030, USA

Citrullinaemia is a human inborn error of metabolism resulting from the deficiency of argininosuccinate synthetase¹. In a previous study of cultured skin fibroblasts from citrullinaemia patients², we showed that the presumed defects in DNA were not detectable by Southern blotting analysis, and that only 2 of 11 cell lines contained detectable enzyme antigen. All citrullinaemia cell lines contained hybridizable mRNA but slight size heterogeneity was noted. Here we report the extension of the analysis of the RNA using S₁ nuclease mapping techniques. Among six cell lines examined, five showed an abnormality of mRNA detectable by S₁ nuclease analysis. The data indicate that a minimum of three out of five non-consanguineous patients represent compound heterozygotes. The S₁ nuclease detectable defects may represent deletions or rearrangements in the genomic DNA, or more probably represent examples of abnormal RNA splicing. The approach used here is useful for molecular analysis of genetic defects, for prenatal diagnosis, and for study of genetic variation.

We used two methods of S₁ nuclease analysis to analyse mRNA from fibroblasts of citrullinaemia patients. For a full linear analysis, a cDNA clone containing the entire length of argininosuccinate synthetase mRNA except about 50 bases of 5' untranslated region was hybridized to the total RNA from cultured fibroblasts in conditions which favoured DNA-RNA hybridization³. After S₁ nuclease digestion, the cDNA fragments were fractionated by electrophoresis, transferred to nitrocellulose paper, and detected with a ³²P-labelled nick-translated cDNA probe. In the S₁ nuclease condition we used, single base additions or substitutions were not detected.

Figure 1 shows results of the full linear S₁ nuclease analysis on RNA from one control and from six citrullinaemia cell lines. All these citrullinaemia cell lines lacked enzyme antigen by immunological analysis. In the 'minus RNA' lane, one major band and some minor bands were detected, identifying fragments not dependent on RNA addition. In the normal control cell line a single band of DNA of approximately 1.5 kilobases (kb) was protected by added RNA. Among the citrullinaemia cell lines, analysis of C.G. showed two protected fragments of 1,243 and 240 bases. The parents of C.G. were first cousins, and S₁ nuclease analysis for both of them showed a heterozygous pattern with a fully protected fragment and a pair of smaller fragments as in C.G. (data not shown). These data suggested that C.G. was homozygous for an allele which produced an RNA with an S₁ nuclease detectable defect. Cell line B.S. had one DNA fragment which was fully protected and two fragments of the same sizes as in C.G. GM63 had one fully protected DNA fragment and two fragments of 1,136 and 191 bases. We interpret the data to indicate that B.S. and GM63 are compound heterozygotes, that is, they each have two different mutant alleles. Cell lines GM1679 and GM3056 are from the same patient² and showed four fragments of 1,084, 875, 496 and 370 bases. The four bands in GM1679 (GM3056) were all of about equal intensity, and we interpret them to represent two pairs of protected DNA fragments from each of two abnormal alleles, although parental cell lines were not available to prove this. Analysis of line GM1044 indicated only a fully protected DNA fragment. It was not clear whether both alleles produced

RNA which fully protected the cDNA or whether one allele did not produce a stable RNA. Analysis of line A.C. indicated two DNA fragments of 997 and 423 bases. Since parental data were not available, A.C. could have two alleles producing similarly abnormal RNA or could have only one allele producing stable RNA.

To orient the sites of the S₁ nuclease detectable lesions in the cDNAs we used a 3' end-labelled cDNA probe for S₁ nuclease analysis. The cDNA was cut at a unique *Hind*III site and was end-labelled at base 232. Figure 2 shows the results of the analysis from the end-labelled site to the 3' end of the mRNA. The RNA from C.G. protected one fragment of 1,013 bases compared with a fragment of 1,320 bases in the control. This located the unprotected site towards the 3' end of the RNA. Analysis of RNA from B.S. revealed a fully protected fragment of 1,320 bases, and a fragment of 1,013 bases, indistinguishable from that seen in C.G. Analysis of cell line GM63 showed only a single fragment of 1,320 bases. The unprotected region in GM63 either was at the 5' end or covered the end-labelled site. By combining information on the size of the unprotected region from Fig. 1 with the failure to detect a smaller DNA fragment in Fig. 2, we conclude that the end-labelled site was within the unprotected region for GM63. GM1679 had two fragments indicating defects at 140 and 645 bases to the 3' side of the end-labelled site. The defect in A.C. was about 190 bases from the end-labelled site.

Figure 3 summarizes our interpretation of the RNA structure in the cell lines being studied. The broken lines in Fig. 3 can

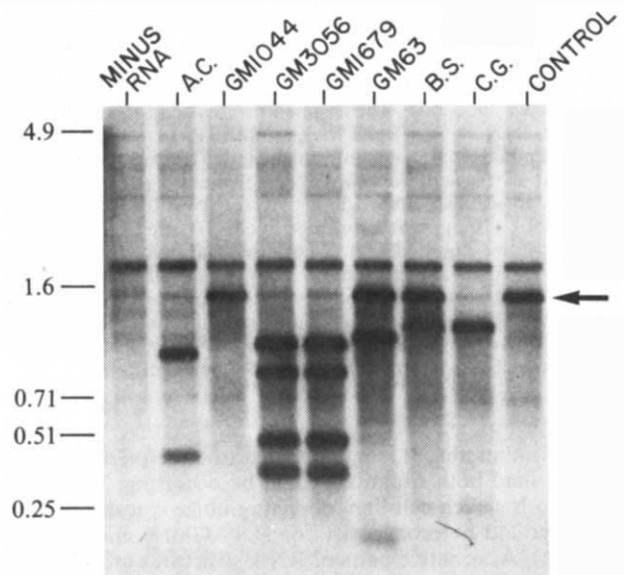


Fig. 1 Full linear S₁ nuclease analysis of a control and citrullinaemia cell lines. The pAS14 clone used for these studies is similar to the previously reported pAS1⁸ except that it contains 70 additional bases at the 5' end. The clone contains only a few bases of the poly(A) region and ~97% of the remainder of the RNA sequence. The 4.9 kb *Hinc*II fragment from pAS14 which contained the 1,550 bases of cDNA with pBR322 sequences flanking both sides was used as cDNA probe. The origins of the patient's cell lines were as described². Total RNA from cultured skin fibroblasts was isolated as described⁹. Total RNA (50 µg) was hybridized with 10 ng of 4.9 kb *Hinc*II fragment and digested with S₁ nuclease as described previously^{10,11}. The precipitated nucleic acid was then resuspended in 20 µl of 0.1 M NaOH, 10 mM EDTA, heated at 68 °C for 10 min, precipitated with ethanol, and denatured by addition of glyoxal¹². The cDNA was fractionated by electrophoresis on a 1.5% agarose gel, transferred to nitrocellulose paper^{13,14} and hybridized with ³²P-nick-translated probe from pAS14 cDNA insert. The arrow indicates the cDNA fragment protected by control RNA. The numbers on the left refer to the position of the migration of the size standards in kilobases.

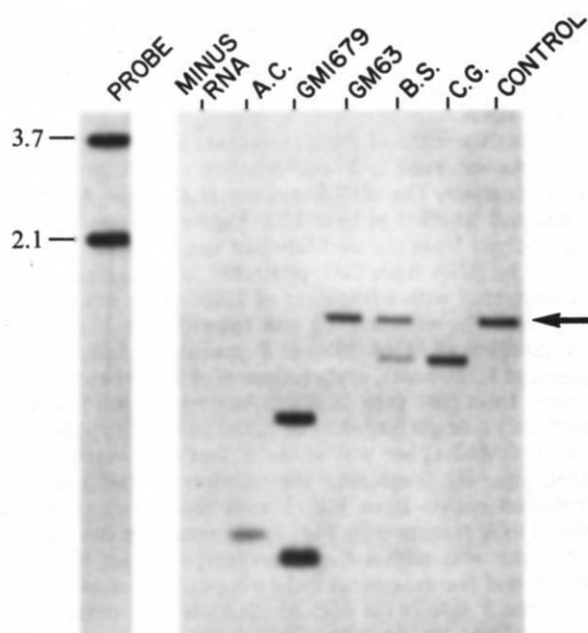


Fig. 2 S_1 nuclease analysis of total RNA from a control and five citrullinaemia cell lines with an end-labelled cDNA probe. The pAS1 plasmid contains 1,480 bases of cDNA inserted into the *Pst*I site of pBR322⁸ and is 70 bases shorter at the 5' end (in respect to RNA) than pAS14. The pAS1 cDNA has a unique *Hind*III site at bases 232–237 which is about 1,320 bases from the 3' end of the cDNA. A sample of 15 μ g of pAS1 plasmid was digested with restriction endonuclease *Hind*III to yield two fragments of 3.7 and 2.1 kb. The fragments were 3' end-labelled using 150 μ Ci each of [α -³²P]dGTP, [α -³²P]dATP, [α -³²P]dCTP (all 3,000 Ci mmol⁻¹, Amersham), unlabelled TTP and the Klenow fragment of *Escherichia coli* DNA polymerase (New England Biolabs) to achieve a specific activity of 1.9×10^7 c.p.m. μ g⁻¹. Total RNA (50 μ g) was hybridized to 2×10^5 c.p.m. of 3' end-labelled probe, digested with S_1 nuclease, and treated with alkali to remove RNA as described in Fig. 1. The cDNA was then reacted with glyoxal and fractionated by electrophoresis on a 1.2% agarose gel. The gel was dried and exposed for autoradiography with an intensifying screen at -70 °C for 4 h. The arrow indicates the cDNA fragment protected by control mRNA.

represent either absent length or substituted sequence (as from an intron) and both situations could be occurring. Two alleles are shown for each cell line, depicting homozygosity for C.G. and compound heterozygosity for B.S., GM63 and GMI679 (GM3056). Alternative pairs of RNA structures are shown for cell lines GM1044 and A.C., since one allele may not produce a stable RNA, as discussed above. All the S_1 nuclease detectable defects fell within the coding region of the cDNA sequence (H.-G. Bock, unpublished data).

Structural defects in the mRNA for argininosuccinate synthetase are present in a large percentage of mutant citrullinaemia alleles. The abnormalities in the RNA document conclusively that the genetic defects in citrullinaemia involve the structural gene for the enzyme. One locus accounts for most and probably all of the mRNA for argininosuccinate synthetase in cultured fibroblasts. The presence of fully protected cDNA fragments is interpreted most simply as (1) the presence of mRNA with small defects, such as single base substitutions or (2) the presence of defects in the extreme 5' region not encompassed by the cDNA clone used. The presence of S_1 nuclease detectable defects in the mRNA could reflect deletions or rearrangements in the DNA. However, base substitutions leading to abnormal RNA splicing appear to be more common than small deletions in the human globin disorders^{4–6}, suggesting that the majority of the S_1 nuclease detectable defects in mRNA

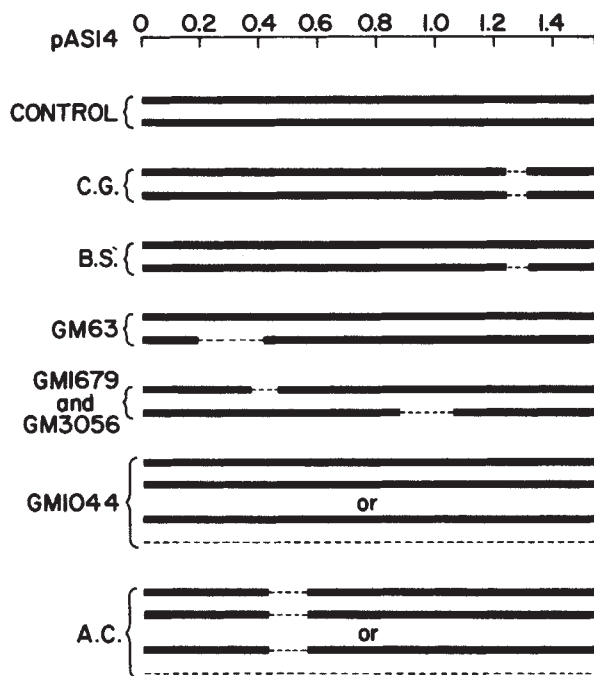


Fig. 3 Schematic representation of mRNA structure in control and citrullinaemia cells. The scale for pAS14 is kilobases and the 5' end of the mRNA is to the left.

reported here may be found to represent RNA splicing abnormalities. DNA sequence analysis of citrullinaemia mutants will require an extensive effort since the expressed gene is large (>60 kb) and contains at least nine introns⁷. The proportion of RNA processing defects as a per cent of all defects in a particular human disease might be proportional to the number of introns, recognizing that chance, the size of the coding region, and the sensitivity of function to amino acid substitutions also would be important.

These studies have shown first, that the state of genetic compound heterozygosity can be demonstrated at the level of mRNA, second, that genetic variation can be monitored conveniently at an RNA level for certain population and linkage studies; and third, the analysis of RNA may be valuable for prenatal diagnosis.

We thank Lynn Loewenstein for preparation of the manuscript. This work was supported by a NIH fellowship for T.-S.S. (GM08199) and by NIH research grants GM27593 and AM25938. Access to patients was enhanced by the support of General Clinical Research Center grant RR-00188.

Received 27 September; accepted 6 December 1982.

- Walser, M. in *Metabolic Basis of Inherited Disease* 5th edn (eds Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S.) 402–438 (McGraw-Hill, New York, 1983).
- Su, T.-S., Bock, H.-G. O., Beaudet, A. L. & O'Brien, W. E. *J. clin. Invest.* **70**, 1334–1339 (1982).
- Casey, J. & Davidson, N. *Nucleic Acids Res.* **4**, 1539–1552 (1977).
- Felber, B. K., Orkin, S. H. & Hamer, D. H. *Cell* **29**, 895–902 (1982).
- Treisman, R., Proudfoot, N. J., Shander, M. & Maniatis, T. *Cell* **29**, 903–911 (1982).
- Ley, T. J., Anagnou, N. P., Pepe, G. & Nienhuis, A. W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4775–4779 (1982).
- Freytag, S. O., Beaudet, A. L. & O'Brien, W. E. *Am. J. hum. Genet.* **34**, 160A (1982).
- Su, T.-S., Bock, H.-G. O., O'Brien, W. E. & Beaudet, A. L. *J. biol. Chem.* **256**, 11826–11831 (1981).
- Wahl, G. M., Padgett, R. A. & Stark, G. R. *J. biol. Chem.* **254**, 8679–8689 (1979).
- Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
- Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
- McMaster, G. K. & Carmichael, G. C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835–4838 (1977).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).

Conformational transitions in oriented fibres of the synthetic polynucleotide poly[d(AT)]·poly[d(AT)] double helix

A. Mahendrasingam, N. J. Rhodes, D. C. Goodwin,
C. Nave*, W. J. Pigram & W. Fuller

Department of Physics, University of Keele,
Staffordshire ST5 5BG, UK

J. Brahms & J. Vergne

Centre National de la Recherche Scientifique, Institut de Biologie
Moléculaire, Université Paris VII, 2 Place Jussieu, Paris V^e, France

The synthetic polynucleotide poly[d(AT)]·poly[d(AT)] is of interest in studies of the relationship between nucleic acid structure and function. In particular, A + T-rich regions in DNA double helices have been invoked as centres for controlling the transcription of genetic information. Here we describe conditions for observing by X-ray fibre diffraction the A, B, C and D conformations of Na-poly[d(AT)]·poly[d(AT)], and for inducing transitions between these conformations. The D form emerges as a particularly stable conformation; once assumed, it persists over a wide range of variation in the relative humidity of the fibre environment. Further, while transitions between the B and D conformations are readily reversible, transitions between A and D are much more complex.

Davies and Baldwin¹ observed that the sodium salt of poly[d(AT)]·poly[d(AT)] could assume not only the crystalline A and semi-crystalline B forms observed for naturally occurring Na-DNAs²⁻⁵ but also a new conformation which was designated D. In the case of Li-poly[d(AT)]·poly[d(AT)], the crystalline B pattern was found but not the semi-crystalline C pattern observed for naturally occurring Li-DNAs⁶. These authors did, however, observe the C pattern for NH₄-poly[d(AT)]·poly[d(AT)]. Davies and Baldwin noted¹ that fibres drawn from the same sample of Na-poly[d(AT)]·poly[d(AT)] gave a variety of patterns—A, D and mixtures of A and D which eventually changed completely to the D form. These observations suggested that the D form is a particularly stable conformation of this synthetic polynucleotide. Arnott *et al.*⁷ reported that the D form was observed in conditions which would have favoured the A form of natural DNAs, that is, a minimum of retained salt in the fibre. Leslie *et al.*⁸ did, however, prepare one fibre for which the A conformation was stable for 6 months even at very high relative humidity (95%). They were unable to identify the conditions which favoured the A and D forms although for one fibre which they described as impure synthetic DNA, they observed the D→A→B transition by increasing the relative humidity of the fibre environment. No indication was given either of the nature of the impurities in this fibre or of the quality of the patterns obtained.

Arnott *et al.*⁷ emphasized that the D pattern from Na-poly[d(AT)]·poly[d(AT)] was very similar to patterns obtained by Mitsui *et al.*⁹ from Na-poly[d(IC)]·poly[d(IC)] and by themselves from poly[d(GC)]·poly[d(GC)]. Mitsui *et al.*⁹ suggested that the patterns from poly[d(IC)]·poly[d(IC)] were best accounted for by a left-handed double helix with Watson-Crick base-pairing and eight nucleotide pairs per helix pitch of 25.01 Å. They found that right-handed models were inferior with respect to either stereochemistry or agreement with the X-ray data compared with the best left-handed models. Arnott *et al.*⁷ dismissed this left-handed model as bizarre and on the basis of a detailed analysis using the linked atom least-squares

refinement technique, they proposed a model for the D conformation of all three complementary poly[d(Pur-Pyr)]·[d(Pur-Pyr)] molecules; this consisted of a right-handed 8-fold double helical structure based on Watson-Crick base-pairing with a sugar pucker and conformational angles resembling the B conformation of natural DNAs.

Gupta *et al.*¹⁰ claim that both the right- and left-handed models which they propose for the D conformation of DNA are in qualitative agreement with the observed diffraction pattern, with agreement indices which are almost identical for the two models and very close to values calculated for the right-handed model proposed by Arnott *et al.*⁷. Recently, Drew and Dickerson¹¹ have described a left-handed model for the D conformation based on Hoogsteen base-pairing¹² with seven nucleotide pairs per helix pitch. They claim that this model is in better agreement with the X-ray fibre diffraction data than previously published models.

Fibres of Na-poly[d(AT)]·poly[d(AT)] were prepared from material synthesized specially for this study or purchased from Boehringer. From previous work on the polynucleotide there was evidence that the conformation assumed was influenced by the ionic content of the fibre. We therefore used preparative procedures which allowed the NaCl in the fibre to be systematically varied. While the NaCl content of a solution of the polynucleotide is readily varied by dialysis, it is more difficult to control the level of salt in precipitated material as there is variability in the amount of salt which is 'brought down' with the polynucleotide. If the polynucleotide is centrifuged rather than precipitated from solution, greater reproducibility is expected in the amount of NaCl associated with the extracted material. For both precipitation and centrifugation, the yield of polynucleotide decreases as the ionic strength of the original solution is reduced. In this study we precipitated the poly[d(AT)]·poly[d(AT)] from ≤0.1 M NaCl and centrifuged it at 50,000 r.p.m. from solutions of ionic strength 0.001–0.05 M NaCl. From measurements on the supernatant it was possible to estimate the amount of NaCl per PO₄⁻ in the extracted material. These estimates are probably unreliable because of non-uniformity in the distribution of NaCl in the gel and precipitate. Nevertheless, this work and a recent parallel study on natural DNAs¹³ represent a much more extensive and systematic analysis of the effect of NaCl on polynucleotide conformation than has been reported previously, except for studies of DNA¹⁴ in which much larger amounts of material were available than is the usual situation for synthetic polynucleotides and indeed for many species of natural DNA.

X-ray fibre diffraction patterns were recorded over a range of relative humidities from 0 to 98%. We observed well-defined X-ray diffraction patterns of the A, B, C and D types from Na-poly[d(AT)]·poly[d(AT)]. The A patterns (Fig. 1a) have a degree of orientation and crystallinity as high as the best defined patterns observed for natural DNA² and much better than any previously reported for Na-poly[d(AT)]·poly[d(AT)]. Two types of B pattern were observed: a semi-crystalline pattern (Fig. 1b) very similar to that observed by Arnott and Selsing¹⁵ from Na-poly(dA)·poly(dT) and designated B', and a semi-crystalline pattern (Fig. 1c) similar to that given by the sodium salt of natural DNAs at high relative humidity. The C patterns did not differ significantly from those observed for natural NaDNAs although they were not as well defined as those recently reported for Na-poly[d(AC)]·poly[d(GT)]¹³. The D patterns (Fig. 1d) showed significantly better orientation and crystallinity than previously reported for this pattern from poly[d(AT)]·poly[d(AT)]. In particular, the orientation was sufficient for the meridional on the eighth layer line to be clearly distinguished from neighbouring reflections when the fibre was tilted appropriately. Furthermore, the strong diffraction on the seventh layer line was observed to be due to 107 and 117 rather than 007.

Fibres estimated to contain one or more Na⁺ and Cl⁻ ion pairs per DNA PO₄⁻ gave patterns dominated by diffraction from salt crystallites. For fibres estimated to contain ~0.6 Na⁺

* Present address: SERC Daresbury Laboratory, Warrington WA4 4AD, UK.

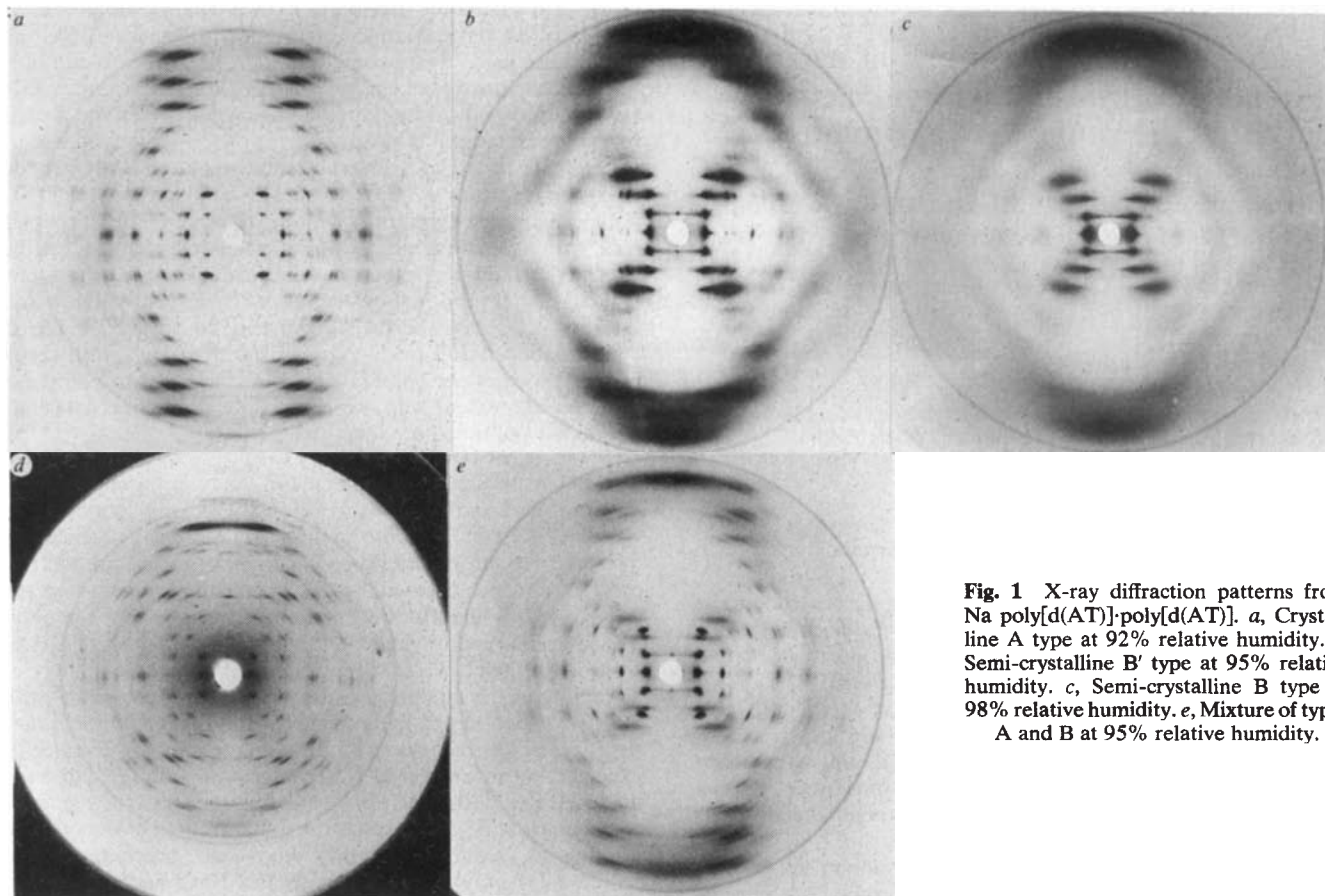


Fig. 1 X-ray diffraction patterns from Na poly[d(AT)]·poly[d(AT)]. *a*, Crystalline A type at 92% relative humidity. *b*, Semi-crystalline B' type at 95% relative humidity. *c*, Semi-crystalline B type at 98% relative humidity. *e*, Mixture of types A and B at 95% relative humidity.

and Cl^- ion pairs per PO_4^- , an A pattern was observed at low relative humidities (typically 32–75%). At higher relative humidities (typically 75–92%) the patterns observed were mixtures of types A and B (Fig. 1e). Patterns in which these two types are so clearly defined within one diffraction pattern have not previously been reported. On prolonged exposure to higher relative humidities (typically 92–95%), the polynucleotide underwent a transition to the D form. Exposure to humidities of 98% resulted in a transition from the D to the B form. Reduction in the relative humidity below 98% resulted in a reversion to the D form which then persisted even when the relative humidity was reduced to 33%. Other fibres having NaCl concentrations in this range assumed the D form over a period of a few months without exposure to high humidities. For fibres estimated to contain smaller amounts of NaCl (typically 0.4 Na^+ and Cl^- ion pairs per PO_4^-), the sequence of transitions as a function of relative humidity was similar except that at low relative humidities the pattern was a mixture of A and C types which changed to an A pattern at relative humidities in the range 66–75%. The transition from an A pattern to a mixture of A and B patterns also occurred at a higher relative humidity. Fibres estimated as containing the smallest amounts of NaCl (typically $<0.2 \text{ Na}^+$ + Cl^- ion pair per PO_4^-) gave C patterns at low relative humidities (typically up to 75% or 92%) above which they changed reversibly into the B form. These fibres exhibited neither the A nor D forms.

In agreement with previous reports, this study shows that the D form is a particularly stable form of Na-poly[d(AT)]·poly[d(AT)]. From our studies it is clear that there is a marked tendency for the polynucleotide to become 'locked into' the D form in which it is resistant to marked changes in the relative humidity of the fibre environment. The assumption of the D form is facilitated by raising the relative humidity of the fibre environment to $\geq 92\%$ when there are moderate levels of NaCl in the fibre and also by leaving the fibre for substantial periods (months) at more moderate humidities. It is important, however, not to over-exaggerate the importance of the D form.

In contrast to earlier reports⁸ we experienced no difficulty in inducing Na-poly[d(AT)]·poly[d(AT)] to assume the A form. Further, the A form in fibres was stable at 75% relative humidity for considerable periods; also the D form was not observed for fibres containing very low levels of NaCl. However transitions from the D form to other forms were only observed after prolonged exposure to high humidities. At low NaCl concentrations and low relative humidities the C form is again the stable form of the sodium salt of the DNA double helix¹³, adding further support to the view that this conformation could be of fundamental biological significance.

The observation in this study of the B' diffraction pattern from fibres of Na-d(AT)·d(AT) is of particular importance as it demonstrates that the occurrence of the B' conformation is not limited to the homopolymer duplex Na-poly(dA)·poly(dT), as has been claimed¹⁵. The observations reported here suggest that the B' conformation could be assumed by all double-helical regions containing only adenine and thymine residues irrespective of their nucleotide sequence.

An important aspect of this study is the evidence we have obtained for the existence within a single fibre of the A, B, C and D conformations with transitions between them as a function of relative humidity. The transitions involving the A, B and D forms are of particular interest. While the B to D transition is observed to be reversible the A to D transition is not. The only transition we have observed from the D form is that at very high humidity into B. It is tempting to speculate that this irreversibility is due to the D form being a left-handed helical structure. If the A and B forms are indeed right-handed as is generally accepted, both the A $\rightarrow$ D and the D $\rightarrow$ B transitions will involve a change of helix sense. A fibre environment might be expected to impose serious constraints on the occurrence of such a profound structural change. Certainly if such changes do occur within a fibre they would be expected to be far more likely when the water content is high or is at least increasing after an increase in the relative humidity of the fibre environment. Transitions involving a change of helix sense

could be important in mechanisms for the manipulation of the genetic information which involve unwinding of the DNA helix. It is therefore important to investigate as intensively as possible the handedness of the D conformation. The X-ray fibre diffraction patterns obtained from the D form in this investigation are much better defined than those previously reported, thus they should allow a definitive determination of the helix sense. We are now pursuing such an analysis.

This work was supported by the SERC (to W.F. and W.J.P.).

Received 1 September; accepted 29 November 1982.

1. Davies, D. R. & Baldwin, R. L. *J. molec. Biol.* **6**, 251–255 (1963).
2. Fuller, W., Wilkins, M. H. F., Wilson, H. R. & Hamilton, L. D. *J. molec. Biol.* **12**, 60–80 (1965).
3. Langridge, R. *et al. J. molec. Biol.* **2**, 38–64 (1960).
4. Arnott, S. & Hukins, D. W. L. *Biochem. biophys. Res. Commun.* **47**, 1504–1509 (1972).
5. Arnott, S. & Hukins, D. W. L. *J. molec. Biol.* **81**, 93–105 (1973).
6. Marvin, D. A., Spencer, M., Wilkins, M. H. F. & Hamilton, L. D. *J. molec. Biol.* **3**, 547–565 (1961).
7. Arnott, S., Chandrasekaran, R., Hukins, D. W. L., Smith, P. J. C. & Watts, L. *J. molec. Biol.* **88**, 523–533 (1974).
8. Leslie, A. G. W., Arnott, S., Chandrasekaran, R. & Ratliff, R. L. *J. molec. Biol.* **143**, 49–72 (1980).
9. Mitsui, Y. *et al. Nature* **228**, 1166–1169 (1970).
10. Gupta, G., Bansal, M. & Sasisekharan, V. *Int. J. biol. Macromolecules* **2**, 368–380 (1980).
11. Drew, H. R. & Dickerson, R. E. *EMBO J.* **1**, 663–667 (1982).
12. Hoogsteen, K. *Acta crystallogr.* **12**, 822–823 (1959).
13. Rhodes, N. J. *et al. Nature* **296**, 267–269 (1982).
14. Cooper, P. J. & Hamilton, L. D. *J. molec. Biol.* **16**, 562–563 (1966).
15. Arnott, S. & Selsing, E. *J. molec. Biol.* **88**, 509–521 (1974).

Cloning and sequence analysis of cDNA for ovine corticotropin-releasing factor precursor

Yasuji Furutani*, Yuuki Morimoto*, Shigeki Shibahara*, Masaharu Noda*, Hideo Takahashi*, Tadaaki Hirose†, Michiko Asai‡, Seiichi Inayama‡, Hidenori Hayashida‡, Takashi Miyata‡ & Shosaku Numa*

* Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan

† Pharmaceutical Institute, Keio University School of Medicine, Tokyo 160, Japan

‡ Department of Biology, Kyushu University Faculty of Science, Fukuoka 812, Japan

Previously, Guillemin and Rosenberg¹ and Saffran and Schally² demonstrated the presence of hypothalamic factors that stimulated the secretion of adrenocorticotrophic hormone (ACTH) by the pituitary gland. Recently, Vale *et al.*^{3,4} have isolated and sequenced an ovine hypothalamic peptide of 41 amino acids, which is believed to represent the major physiological corticotropin-releasing factor (CRF) (reviewed in refs 5, 6). Available data suggest that hypothalamic CRF enhances both the synthesis and secretion of ACTH and related peptides such as β -endorphin and β -lipotropin (β -LPH) (reviewed in ref. 6), which are all derived from the common precursor, termed ACTH- β -LPH precursor or preproopiomelanocortin (reviewed in ref. 7). Because CRF mediates the neural control of the pituitary-adrenocortical system, the characterization of its biosynthetic precursor and the gene encoding it is essential for understanding the molecular mechanism underlying the endocrine response to stress. We have now cloned DNA sequences complementary to the ovine hypothalamic mRNA encoding the CRF precursor (referred to hereafter as prepro-CRF). The nucleotide sequence of the cloned cDNA, reported here, has revealed the primary structure of prepro-CRF. The carboxyl end represents the CRF sequence preceded by the tetrapeptide, Arg-Lys-Arg-Arg, and followed by the dipeptide, Gly-Lys. Comparison of the amino acid sequence of prepro-CRF with those of the ACTH- β -LPH precursor and the arginine vasopressin-neurophysin II precursor⁸ suggests that these precursor proteins may be evolutionarily related.

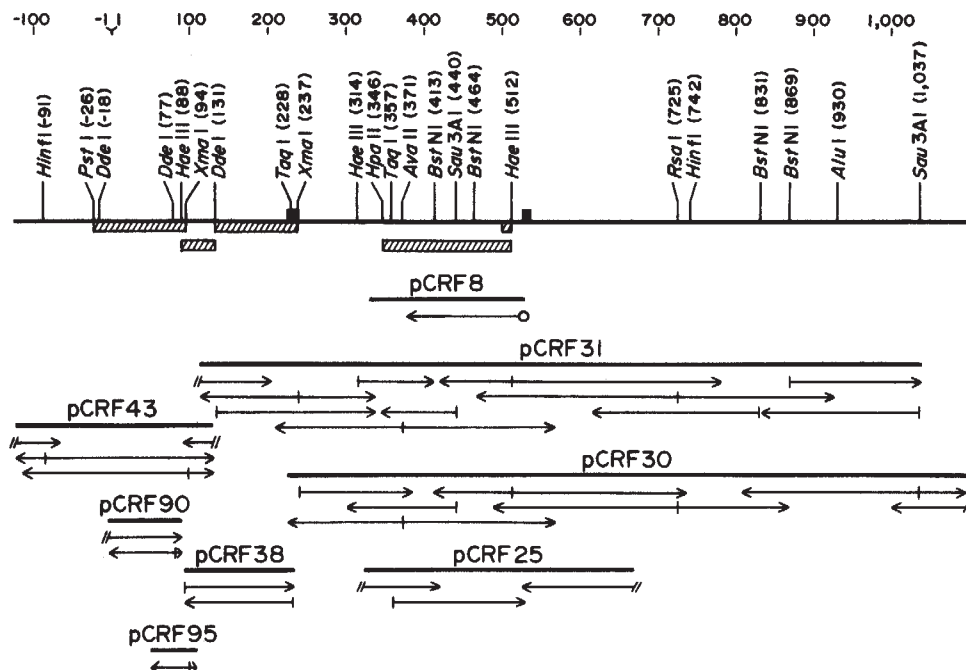
Experimental details of cloning cDNA for prepro-CRF are described in Fig. 1 legend. A library of cDNA clones derived from ovine hypothalamic poly(A) RNA was constructed using the plasmid DNA vector elaborated by Okayama and Berg⁹. Two oligodeoxyribonucleotides, 5'-GC^TTTNGTCAT^TTC-3' and 5'-TGNGC^TTG^TTG-3', were synthesized by the triester method^{10,11}, and each consisted of two pools containing A/T or G/C for N, which represents a mixture of A, T, G and C. These oligodeoxyribonucleotides represent all possible cDNA sequences that correspond to the pentapeptide sequence Glu-Met-Thr-Lys-Ala (amino acid residues 167–171 in Fig. 2, excluding the third nucleotide of the Ala codon) and the tetrapeptide sequence Gln-Gln-Ala-His (amino acid residues 176–179 in Fig. 2, excluding the third nucleotide of the His codon), respectively, of ovine CRF (ref. 3). Our initial attempts to isolate cDNA clones that hybridized with both oligodeoxyribonucleotide probes were unsuccessful, despite ~1,000,000 transformants from the cDNA library being screened.

An alternative approach was therefore used to enrich cDNA for prepro-CRF by specific priming of the reverse transcription of prepro-CRF mRNA using the synthetic undecamer described above; ovine hypothalamic poly(A) RNA served as template. The transcripts formed by elongation of the synthetic primer were converted to double-stranded cDNA, which was cloned in plasmid pBR322. The resulting clones were screened for cDNA encoding prepro-CRF by hybridization with the above-mentioned synthetic tetradecamer probe, which corresponds to the pentapeptide sequence located upstream of the tetrapeptide sequence represented by the undecamer primer. Thus, four hybridization-positive clones were isolated from ~720,000 transformants. Nucleotide sequence analysis¹² (Fig. 1) revealed that one of these clones, pCRF8, harboured a cDNA sequence encoding most of the CRF structure.

Because clone pCRF8 evidently did not contain the entire protein-coding sequence, we re-screened the original cDNA library constructed by using the Okayama-Berg vector, this time using as hybridization probe the *Hpa*II(346)–*Hae*III(512) fragment (Fig. 1) derived from this clone. Of ~1,500,000 transformants, five hybridization-positive clones were isolated and classified by restriction endonuclease mapping into three groups, each having a different 3' terminus (see below). One clone from each group (clones pCRF31, pCRF30 and pCRF25) was subjected to nucleotide sequence analysis¹² according to the strategy indicated in Fig. 1.

Because these clones also did not carry the entire protein-coding sequence, a further attempt was made to clone DNA sequences complementary to the upstream region of prepro-CRF mRNA. The oligodeoxyribonucleotide 5'-GGGTCTCATCGAGGT-3', which is complementary to the mRNA sequence of residues 224–238 (Fig. 2) in clones pCRF30 and pCRF31, was synthesized by the triester method¹¹ and used for specific priming of the reverse transcription of prepro-CRF mRNA. The resulting cDNA was cloned in plasmid pBR322 and screened by hybridization with the *Dde*I(131)–*Xma*I(237) fragment (Fig. 1) derived from clone pCRF31. One of the five hybridization-positive clones isolated from ~300,000 transformants, that is, pCRF38, whose cDNA insert extended beyond the *Dde*I(131) site, was subjected to DNA sequencing¹². Because the 5' end of the cDNA insert of clone pCRF38 barely extended beyond that of clone pCRF31, we re-screened the same ~300,000 transformants, using the *Hae*III(88)–*Dde*I(131) fragment derived from clone pCRF38 as a hybridization probe. Thus, one hybridization-positive clone (pCRF43) was isolated, and its cDNA insert was sequenced¹² (Fig. 1). About 250,000 additional transformants were then screened by hybridization with the *Pst*I(–26)–*Xma*I(94) fragment derived from clone pCRF43 to yield five hybridization-positive clones. Restriction endonuclease mapping indicated that none of these clones carried a cDNA sequence extending beyond the 5' end of the cDNA insert of clone pCRF43. Two of these, pCRF90 and pCRF95, were subjected to DNA sequencing¹² (Fig. 1).

Fig. 1 Strategy for sequencing cloned cDNA encoding prepro-CRF. The restriction map shows only the relevant restriction endonuclease sites, which are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage (for the nucleotide numbers see Fig. 2). The poly(dA)·poly(dT) tract and the poly(dG)·poly(dC) tails are not included in the restriction map, except for the poly(dG)·poly(dC) tail at the 5' end of pCRF8; the location of this terminus is approximate. The sequences used for specific priming of reverse transcription are indicated by closed boxes, and those used as hybridization probes for selecting clones by hatched boxes. DNA sequencing was carried out by the procedure of Maxam and Gilbert²²; for 3'-end labelling, see ref. 34. The direction and extent of sequence determinations are shown by horizontal arrows under each clone used; the sites of 5'-end labelling are indicated by short vertical lines at the end of the arrows, and the site of 3'-end labelling (*Pst*I site) by an open circle. The slash marks at the ends of arrows mean that the site of 5'-end labelling was located on the vector DNA^{9,35,36} as follows (the numbers in parentheses indicate the distance in nucleotides between the site of labelling and the *Pst*I site (on the 5' side) or *Rsa*I site (on the 3' side) flanking the cDNA insert): *Rsa*I (22) for pCRF31; *Rsa*I (0) for pCRF30; *Rsa*I (left mark) (22) and *Rsa*I (right mark) (0) for pCRF25; *Alu*I (left mark) (48) and *Alu*I (right mark) (56) for pCRF43; *Sau*3A1 (66) for pCRF90. Details of the sequencing procedures have been described previously¹⁵.



Methods: Total RNA was extracted³⁷ from ovine hypothalami, and poly(A) RNA was isolated by oligo(dT)-cellulose chromatography³⁸. For the isolation of clone pCRF8, single-stranded cDNA was synthesized by avian myeloblastosis virus reverse transcriptase (provided by Dr J. W. Beard), using 200 µg of poly(A) RNA as template and the synthetic oligodeoxyribonucleotide primer specified in the text. The cDNA synthesized was converted to double-stranded cDNA, which was cloned in plasmid pBR322 by inserting it into the *Pst*I site using poly(dG)·poly(dC) homopolymeric extensions; the procedures used were as described previously³⁹. *Escherichia coli* χ 1776 (ref. 40) or HB101 (ref. 41) was used for transformation⁴². Tetracycline-resistant transformants were screened⁴³ by hybridization at 43 °C with the 5'-³²P-labelled synthetic oligodeoxyribonucleotide probe specified in the text. For the isolation of clones pCRF31, pCRF30 and pCRF25, a cDNA library was constructed by the method of Okayama and Berg⁷ using 2.0 µg of poly(A) RNA and 1.4 µg of the vector-primer DNA. Ampicillin-resistant transformants were screened⁴³ by hybridization at 60 °C with the probe mentioned in the text (labelled by nick-translation⁴⁴ with [α -³²P]dCTP). For the isolation of clones pCRF38, pCRF43, pCRF90 and pCRF95, the synthetic oligodeoxyribonucleotide primer specified in the text was elongated by reverse transcriptase using 250 µg of poly(A) RNA as template. The subsequent procedures were the same as described above, except that hybridization for selecting clones was done at 50 °C (pCRF43) or 60 °C (pCRF38, pCRF90 and pCRF95) with the probes mentioned in the text (labelled by nick-translation, except for the *Hae*III(88)-*Dde*I(131) fragment, which was 5'-end-labelled).

Figure 2 shows the primary structure of ovine prepro-CRF mRNA deduced from the cDNA sequence. The nucleotide sequence of the whole protein-coding region was determined for at least two clones. The sequence of nucleotide residues 442-564 corresponds precisely to the amino acid sequence determined for CRF (amino acid residues 148-188)³. The amino-terminus of CRF is preceded by the sequence Arg-Lys-Arg-Arg, which apparently represents the site of proteolytic processing¹³. The carboxy-terminus of CRF is connected to the sequence Gly-Lys, and the codon specifying this lysine residue is followed by the translational termination codon, UGA. The glycine residue following the CRF sequence probably serves as a donor of the amide group for the carboxy-terminal alanine residue of CRF (ref. 3), as suggested for other peptide hormones terminating in an α -amide group^{8,14-19}. This concept has recently been proved by the presence of a pituitary enzyme that converts peptides terminating in glycine to the corresponding des-glycine peptide amide²⁰. Interestingly, the CRF sequence is followed by Gly-Lys, which may also act as a signal for enzymatic amidation. It is possible, however, that the amidation occurs after elimination of the terminal lysine residue by carboxypeptidase B-like activity.

The translational initiation site is tentatively assigned to the methionine codon AUG at positions 1-3, which is the first AUG triplet within the mRNA sequence deduced, because the sequence of the 24 amino acid residues starting with this methionine (residue 1) exhibits a feature characteristic of the signal peptide at the amino-terminal end of secretory proteins²¹. The signal peptide generally contains a region rich in hydro-

phobic amino acids with large side chains in its central portion and terminates in a residue having a small neutral side chain¹³ (for example, alanine, glycine or serine). Therefore, a possible site for cleavage of the signal peptide of prepro-CRF seems to exist after the alanine residue at position 24 (or after the serine residue at position 19 or 27).

The individual cDNA clones used for sequence determinations exhibit five nucleotide differences (see Fig. 2 legend). One of the three differences found in the protein-coding region results in the replacement of the lysine residue at position 127 by a glutamic acid residue, whereas the other two differences lead to synonymous codons. The observed nucleotide differences may be due to polymorphism^{22,23} in the ovine prepro-CRF gene because the template RNA used for *in vitro* cDNA synthesis was derived from the pooled hypothalami of many individuals. However, possible errors occurring during *in vitro* cDNA synthesis or DNA cloning²⁴ cannot be excluded.

The structure of ovine prepro-CRF is illustrated schematically in Fig. 3. The precursor protein consists of 190 amino acids including a putative signal peptide. The cryptic portion of ovine prepro-CRF contains, in addition to the Arg-Lys-Arg-Arg sequence immediately preceding CRF, two additional paired basic amino acid residues (Arg-Arg at positions 116 and 117 and Lys-Arg at positions 127 and 128); the lysine residue at position 127 is subject to a replacement by a glutamic acid residue (see above). It seems plausible to assume that the prohormone is proteolytically cleaved at one or both of these sites to produce novel peptides, which may function synergistically or coordinately with CRF.

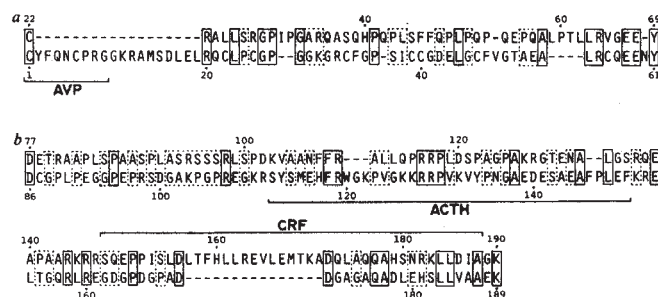


Fig. 4 Alignment of the amino acid sequence of ovine prepro-CRF with the sequence of the bovine arginine vasopressin-neurophysin II precursor (a) and the human ACTH- β -LPH precursor (b). The one-letter amino acid notation is used. In a, amino acid residues 22–69 of ovine prepro-CRF (top) are aligned with residues 1–61 of the bovine arginine vasopressin-neurophysin II precursor⁸ (bottom). In b, residues 77–190 of ovine prepro-CRF (top) are aligned with residues 86–189 of the human ACTH- β -LPH precursor⁴⁵ (bottom); the lysine residue has been taken for position 127 of prepro-CRF (see text and Fig. 2). Amino acid numbers (see Fig. 2 and refs 8, 45) are given, and the positions of CRF, arginine vasopressin (AVP) and ACTH are indicated. Sets of identical residues are enclosed with solid lines, and sets of residues considered to be favoured amino acid substitutions with dotted lines. Favoured amino acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W; C (see ref. 46). (Gaps (-) have been inserted to achieve maximum similarity. Statistical tests were made to assess the extent of similarity between the sequences compared. The score per residue for the aligned sequences (s) was calculated, using the scoring matrix MDM₇₀ (ref. 47); gaps were counted as one substitution regardless of their length, and a score of -60 was assigned for a gap. The score, s, was compared with the highest possible scores obtained by aligning pairs of randomized sequences having the same amino acid compositions as those of the real sequences; the highest possible score for a pair of randomized sequences was achieved by a computer algorithm⁴⁸. Thirty such pairs of randomized sequences were scored, and the mean (m) and standard deviation (σ) of the scores were calculated. The alignment score A, defined as $A = (s - m)/\sigma$ (ref. 47), was then evaluated. The values obtained were as follows: for a, $s = 3.49$, $m = -1.41$, $\sigma = 2.05$ and $A = 2.39$ ($P = 0.0084$); for b, $s = 7.08$, $m = 2.42$, $\sigma = 1.48$ and $A = 3.15$ ($P = 0.0008$).

arginine vasopressin potentiates the ACTH-releasing activity of CRF³³.

We thank Dr Paul Berg and Dr Hiroto Okayama for providing us with their high-efficiency cloning system. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

Received 23 November; accepted 2 December 1982.

- Guillemin, R. & Rosenberg, B. *Endocrinology* **57**, 599–607 (1955).
- Saffran, M. & Schally, A. V. *Can. J. Biochem. Physiol.* **33**, 408–415 (1955).
- Vale, W., Spiess, J., Rivier, C. & Rivier, J. *Science* **213**, 1394–1397 (1981).
- Spiess, J., Rivier, J., Rivier, C. & Vale, W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6517–6521 (1981).
- Fink, G. *Nature* **294**, 511–512 (1981).
- Yasuda, N., Greer, M. A. & Aizawa, T. *Endocr. Rev.* **3**, 123–140 (1982).
- Numa, S. & Nakanishi, S. *Trends biochem. Sci.* **6**, 274–277 (1981).
- Land, H., Schütz, G., Schmale, H. & Richter, D. *Nature* **295**, 299–303 (1982).
- Okayama, H. & Berg, P. *Molec. cell. Biol.* **2**, 161–170 (1982).
- Hirose, T., Crea, R. & Itakura, K. *Tetrahedron Lett.* **28**, 2449–2452 (1978).
- Ito, H., Ike, Y., Ikuta, S. & Itakura, K. *Nucleic Acids Res.* **10**, 1755–1769 (1982).
- Maxam, A. M. & Gilbert, W. *Methods Enzym.* **65**, 499–560 (1980).
- Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J. & Tager, H. S. *Ann. N. Y. Acad. Sci.* **343**, 1–16 (1980).
- Suchanek, G. & Kreil, G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 975–978 (1977).
- Nakanishi, S. *et al. Nature* **278**, 423–427 (1979).
- Shibasaki, T., Ling, N. & Guillemin, R. *Biochem. biophys. Res. Commun.* **96**, 1393–1399 (1980).
- Seidah, N. G., Rochemont, J., Hamelin, J., Benjannet, S. & Chrétien, M. *Biochem. biophys. Res. Commun.* **102**, 710–716 (1981).
- Amara, S. G., David, D. N., Rosenfeld, M. G., Roos, B. A. & Evans, R. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4444–4448 (1980).
- Yoo, O. J., Powell, C. T. & Agarwal, K. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1049–1053 (1982).
- Bradbury, A. F., Finnie, M. D. A. & Smyth, D. G. *Nature* **298**, 686–688 (1982).
- Blöbel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 852–862 (1975).
- Maniatis, T., Kee, S. G., Efstratiadis, A. & Kafatos, F. C. *Cell* **8**, 163–182 (1976).
- Efstratiadis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10**, 571–585 (1977).
- Browne, J. K. *et al. Science* **195**, 389–391 (1977).
- Grantham, R., Gautier, C., Gouy, M., Mercier, R. & Pavé, A. *Nucleic Acids Res.* **8**, r49–r62 (1980).
- Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
- Setzer, D. R., McGrogan, M., Nunberg, J. H. & Schimke, R. T. *Cell* **22**, 361–370 (1980).
- Tosi, M., Young, R. A., Hagenbüchle, O. & Schibler, U. *Nucleic Acids Res.* **9**, 2313–2323 (1981).

- Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350–5354 (1977).
- Beckmann, S. L., Chikaraishi, D. M., Deeb, S. S. & Sueoka, N. *Biochemistry* **20**, 2684–2692 (1981).
- Kaplan, B. B., Schachter, B. S., Osterburg, H. H., de Vellis, J. S. & Finch, C. E. *Biochemistry* **17**, 5516–5524 (1978).
- Bugnon, C., Fellmann, D., Gouget, A. & Cardot, J. *Nature* **298**, 159–161 (1982).
- Gillies, G. E., Linton, E. A. & Lowry, P. J. *Nature* **299**, 355–357 (1982).
- Tu, C.-P. D. & Cohen, S. N. *Gene* **10**, 177–183 (1980).
- Sutcliffe, J. G. *Nucleic Acids Res.* **5**, 2721–2728 (1978).
- Reddy, V. B. *et al. Science* **200**, 494–502 (1978).
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
- Noda, M. *et al. Nature* **295**, 202–206 (1982).
- Curtiss, R. III *et al. in Recombinant Molecules, Impact on Science and Society* (eds Beers, R. F. & Bassett, E. G.) 45–56 (Raven, New York, 1977).
- Boyer, H. W. & Roulland-Dussoix, D. *J. molec. Biol.* **41**, 459–472 (1969).
- Wahl, G. M., Padgett, R. A. & Stark, G. R. *J. biol. Chem.* **254**, 8679–8689 (1979).
- Hanahan, D. & Meselson, M. *Gene* **10**, 63–67 (1980).
- Weinstock, R., Sweet, R., Weiss, M., Cedar, H. & Axel, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1299–1303 (1978).
- Takahashi, H., Teranishi, Y., Nakanishi, S. & Numa, S. *FEBS Lett.* **135**, 97–102 (1981).
- Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 3, 345–352 (National Biomedical Research Foundation, Silver Spring, Maryland, 1978).
- Schwartz, R. M. & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 3, 353–358 (National Biomedical Research Foundation, Silver Spring, Maryland, 1978).
- Sankoff, D. *Proc. natn. Acad. Sci. U.S.A.* **69**, 4–6 (1972).

Prediction of super-secondary structure in proteins

W. R. Taylor & J. M. Thornton

Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, UK

Various methods for the prediction of secondary structure from amino acid sequence can consistently achieve on average 60% accuracy when tested for several proteins^{1–3}. Improvement on this value has proved difficult, despite increasing the size of the data set and refining predictive techniques⁴. The difficulty almost certainly derives from the influence of long-range interactions and the restrictions required to attain favourable protein topologies. We describe here a novel approach to structure prediction from amino acid sequence based on the recognition of super-secondary structure. The structure we initially consider is the $\beta\alpha\beta$ unit, which consists of two parallel β -strands connected by an α -helix. From an analysis of all known $\beta\alpha\beta$ units, an ideal secondary structure sequence was derived. This was used as a template to locate probable $\beta\alpha\beta$ sequences in a standard secondary structure prediction. The method correctly predicted the location of 70% of the $\beta\alpha\beta$ units in 16 β/α type proteins. This led to a 7.5% average improvement over the original secondary structure prediction.

With the increasing number of known protein structures it has become apparent that there are well-defined sub-assemblies of local secondary structure which dominate the tertiary folds of many globular proteins⁵. The interactions involved are of intermediate range as contacts between sequentially adjacent units of secondary structure predominate (see Fig. 1a). The super-secondary structures identified so far include the $\beta\alpha\beta$ unit (found in the β/α family of proteins), the β -hairpin and β -meander structures (found in many proteins, but especially the all- β family), and the α - α hairpin. Here we consider the $\beta\alpha\beta$ unit, which is widespread and well defined. It is also particularly difficult to predict by conventional methods because the β and α predictions frequently overlap⁵.

We analysed 62 $\beta\alpha\beta$ units of known sequence and structure from 18 different proteins in the Cambridge data bank⁶. From these experimental data we calculated average lengths of helix, strand and coil regions and constructed an 'ideal' $\beta\alpha\beta$ unit (see Fig. 1b). To gain an impression of the homogeneity of the $\beta\alpha\beta$ structures, the observed $\beta\alpha\beta$ sequences were scaled (expanded or contracted) to produce a maximum degree of correspondence with the ideal sequence. Over the adjacent stranded $\beta\alpha\beta$ s the scale factor ranged from 0.63 to 1.32 with a mean ($\pm$ s.d.) of 1.00 ± 0.17 . Combining the scaled observed sequences we produced a histogram of frequency of secondary structure occur-

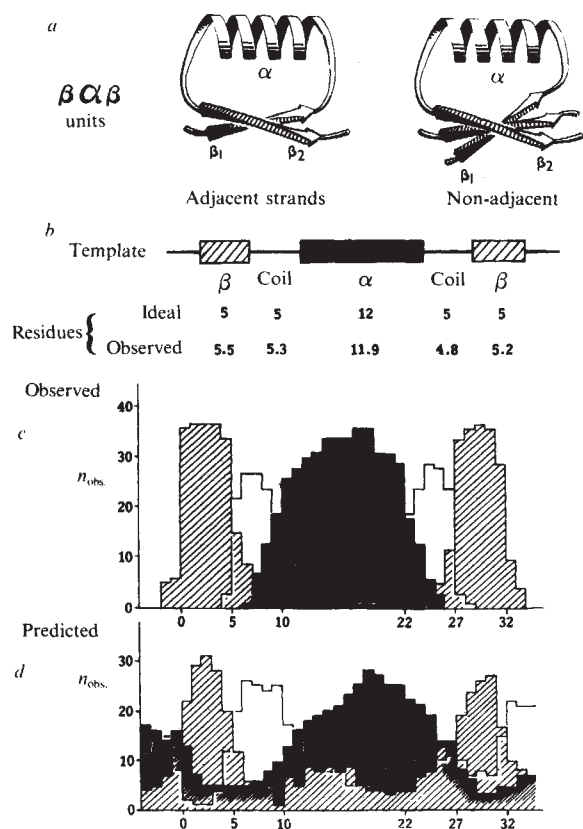


Fig. 1 *a*, Schematic representation of two $\beta\alpha\beta$ units. The α -helix lies 10 Å above a twisted β -sheet, bridging two parallel strands by a right-handed connection. The figures illustrate a $\beta\alpha\beta$ in which the strands lie adjacent in the β -sheet and a $\beta\alpha\beta$ where the strands are not adjacent. *b*, The $\beta\alpha\beta$ template derived from the average observed lengths of secondary structure in $\beta\alpha\beta$ units. The idealized lengths and observed average lengths of each structural element are shown below the template. *c*, Frequency histogram of secondary structure occurrence in observed $\beta\alpha\beta$ units scaled to correspond with the template. The structures are: solid, α -helix; cross-hatched, β -strand; and open coil. *d*, Frequency histogram as in *c* for the secondary structure predicted by the method of Garnier *et al.*². Each $\beta\alpha\beta$ sequence was scaled and aligned as in *c*.

rence for each position on the ideal sequence. The histogram (Fig. 1c) is for $\beta\alpha\beta$ units having their two strands lying adjacent in the β -sheet. The corresponding histogram for the $\beta\alpha\beta$ s with non-adjacent strands is slightly less well resolved. The sharp definition of the different secondary structure elements in Fig. 2c indicates that one scaling parameter is sufficient to model most observed $\beta\alpha\beta$ s starting from the ideal sequence.

The secondary structure predicted by the method of Garnier *et al.*² was also combined into a frequency histogram (Fig. 1d). This combination used the scaling parameters derived from the fit of the observed structures, thus yielding the two histograms (Fig. 1c, d) directly comparable. As expected, the predicted regions, although clear, are less well defined than the observed. This 'blurring' was especially pronounced in the prediction of non-adjacent stranded $\beta\alpha\beta$ s.

The predictive method is based on finding the best positions along the protein sequence for a scaled ideal $\beta\alpha\beta$ template. This is done by finding the areas under the predicted secondary structure probability curves for each type of structure in the region where it corresponds to the scaled ideal sequence. The product of these areas is then taken as the goodness-of-fit (F). Thus if the alignment of the ideal sequence corresponds with peaks in the structure prediction, F is high. However, if any of the required elements is not present, the F value tends towards zero. An F value is calculated for different scaling factors at every position on the sequence and the maximum F values indicate good $\beta\alpha\beta$ locations. (Details will be published elsewhere.)

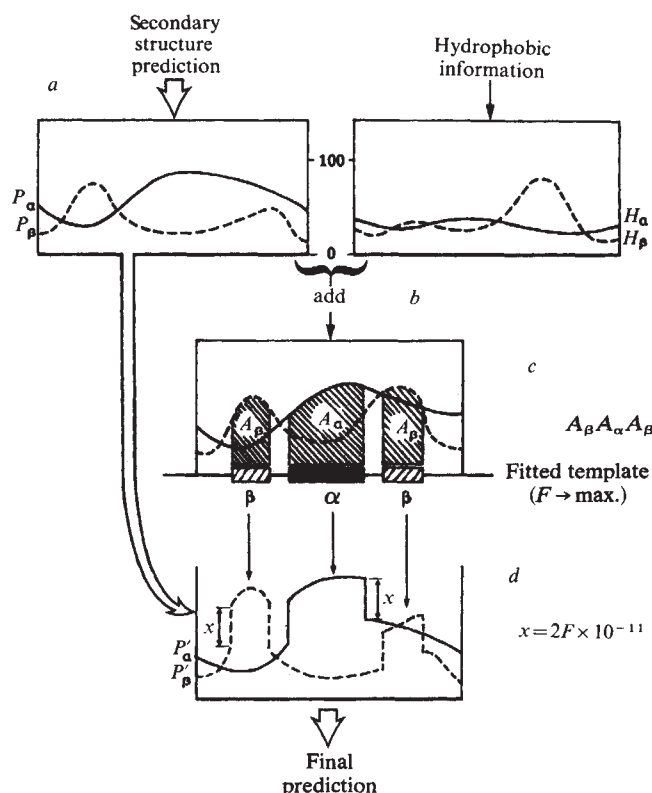


Fig. 2 Outline of the predictive method. *a*, Probability profiles (P_α and P_β) are generated by a standard predictive method (see refs 1, 2). The highest curve determines the predicted state. *b*, Information derived from specific patterns of hydrophobic residues is added to the profiles from *a*. This is based on a hydrophobic score ranging from -2 for a charged residue (hydrophilic) to 2 for large aliphatic and aromatic residues (hydrophobic). β -Strands were identified from the average local hydrophobicity (H_β) and α -helices from an average over residues i , $i+3$, $i+4$ and $i+7$ (H_α). *c*, The $\beta\alpha\beta$ template is scaled (over the entire range of the observed $\beta\alpha\beta$ s) and aligned with the combined profiles to maximize the goodness-of-fit (F). F is the product of the areas (A_α , A_β) under each profile in the range of their corresponding template sections. *d*, A new secondary structure prediction is generated from the original (*a*) by raising the profile levels by 'x' in the region of their corresponding template section. The height x is proportional to F and has an average height of the standard deviation of the original prediction profiles. As in *a*, the highest curve determines the predicted state. For clarity the turn regions were not considered in the above figures. Because of the asymmetry in the predicted turn profile the areas under both turn regions were added, giving A_t . Thus, in practice, $F = A_\beta A_\alpha A_\beta A_t$.

The application of this basic method to the location of $\beta\alpha\beta$ s and the refinement of secondary structure prediction are outlined in Fig. 2, and summarized as follows. (1) A standard secondary structure prediction method (for example, that of Chou and Fasman¹ or Garnier *et al.*²) is used to generate the helical (P_α), extended (P_β) and turn/coil (P_t) probability profiles from the sequence. (2) Additional information on the distribution of hydrophobic residues, as specifically required in the $\beta\alpha\beta$ unit, is added to these probabilities⁷. Typically, this contribution is the same order of magnitude as the original P_α and P_β . (3) The ideal $\beta\alpha\beta$ template, which can expand and contract, is fitted to the β , α and turn profiles for each position on the sequence and the maximum (F) is found. The fits having the highest F , which do not cause β and α regions to overlap, are taken as the most likely $\beta\alpha\beta$ locations. (4) For the refinement of the secondary structure prediction, the P_α , P_β and P_t profiles are weighted by F (in the Garnier *et al.* method² this is equivalent to a local change of decision constant) and a new secondary structure prediction is obtained.

The above method was applied to 16 $\beta\alpha\beta$ proteins of known sequence and structure. In these structures 80% of the adjacent

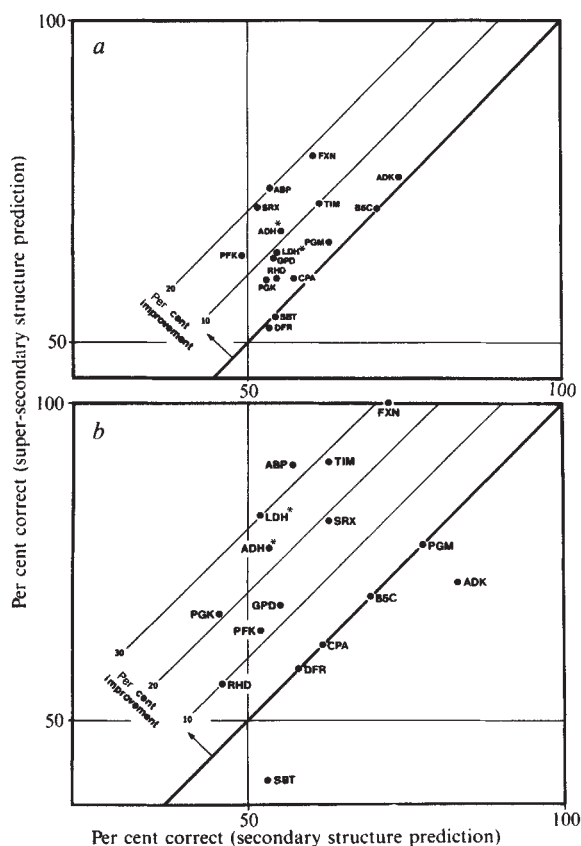


Fig. 3 *a, b* Show the effect of considering super-secondary structure. The accuracy of secondary structure prediction is plotted against prediction accuracy after super-secondary structure reinforcement (super-secondary structure prediction) for each protein. Thus, proteins above the heavy diagonal line are improved by the method, while those below are less well predicted. *a* Shows the per cent of residues predicted in the correct state; *b* shows the per cent of correctly aligned secondary-structure elements (see text and Fig. 4 for details). The proteins considered were: ABP, L-arabinose-binding protein; ADH, liver alcohol dehydrogenase; ADK, adenylate kinase; B5C, cytochrome *b*₅; CPA, carboxypeptidase A; DFR, dihydrofolate reductase; FXN, flavodoxin; GPD, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; RHD, rhodanase; SBT, subtilisin BPN; SRX, thioredoxin; TIM, triose phosphate isomerase. * Nucleotide-binding domains only.

stranded $\beta\alpha\beta$ units were correctly located and 50% of the non-adjacent $\beta\alpha\beta$ s located. This difference again reflects the less regular structure of the non-adjacent $\beta\alpha\beta$ units. The predicted $\beta\alpha\beta$ units were then used to modify the original secondary structure prediction. In this new prediction the percentage of residues predicted in the correct state was compared with the percentage correct before the application of super-secondary reinforcement. These differences are plotted in Fig. 3*a*. An average improvement of 7.5% was observed with some predictions improving by almost 20%. The overall accuracy of 67% can be analysed for each structural component by the correlation measure (*C*) recommended by Matthews⁸. This gave $C_\alpha = 0.50$, $C_\beta = 0.47$ and $C_{\text{coil}} = 0.39$. These values constitute a 0.11 average improvement of the coefficient. A few proteins do not benefit from the method; in general these contain the less regular non-adjacent $\beta\alpha\beta$ units.

To identify proteins to which our method can be applied with benefit, we used the method of Garnier *et al.*² to predict the structural class of 54 proteins from the Cambridge data bank. From the predicted secondary structure composition, 32 proteins (including all those in Fig. 3) were identified as likely β/α or $\beta + \alpha$ type proteins. These were divided into two groups, defined as: probable β/α type proteins ($\alpha > 35\%$ and $\beta > 15\%$) and possible β/α type proteins ($15\% > \alpha > 35\%$ and $\beta > 15\%$).

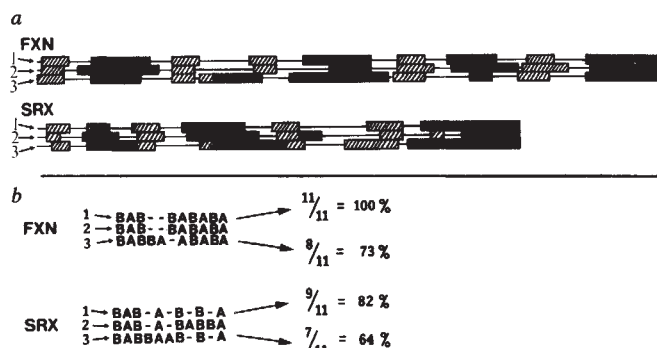


Fig. 4 *a*, The secondary structure of two proteins (FXN and SRX). Each sequence consists of three lines: (1) super-secondary structure prediction; (2) observed secondary structure; (3) secondary-structure prediction (Garnier *et al.*²). Solid blocks represent α -helices; cross-hatched blocks, β -strands; and connecting lines, coil. Helices of <5 residues and strands <3 residues have been omitted. *b*, The sequences in *a*, represented by secondary structure only. A, α -helix; B, β -strand. The percentages of structure correct in the two predicted sequences (that is, $1=2$ and $2=3$) are calculated.

In view of this assignment the P_α and P_β probabilities were readjusted using 'decision constants' (see Garnier *et al.*²) before our method was applied. The group of 17 probable β/α proteins contains 13 of the proteins from Fig. 3. Over this subset (including all catalytic domains) the average improvement obtained by our method was 8.7%. The remaining four proteins (two all- α and two $\beta + \alpha$) suffered a corresponding (9.5%) worsening of prediction accuracy. We hope, however, that physical methods (for example, circular dichroism⁹ or Raman spectroscopy¹⁰) would easily identify all- α proteins and perhaps even $\beta + \alpha$ proteins. Applying our method to the second group (possible β/α proteins), we found those in Fig. 3 improved slightly by 2.7% while the remainder were largely unaffected (0.4% improvement).

Figure 4*a* shows the effect of the method on two small proteins. These sequences illustrate the general observation that often the form of the secondary structure is roughly correct but the strands and helices are slightly displaced from the correct positions. To obtain a better measure of how well the pattern of secondary structure was predicted, an algorithm was devised which assessed the accuracy of the prediction at the level of secondary structure. This automatic process found the percentage of correctly aligned structure elements (with at least one residue overlap), neglecting strands of less than three residues and helices of less than five residues (see Fig. 4*b*). The effect of super-secondary structure prediction as measured by this procedure is shown in Fig. 3*b*; clearly, a third of the proteins improve by more than 20%, with an average improvement of 11.5%.

The approach described here is seen as a general method which can be applied to the results of any predictive scheme and can easily be adapted to search for any pattern of secondary structure. Furthermore, it provides a framework into which increasingly higher level restraints may be incorporated, such as the recognition of Rossmann folds¹¹ or Greek key structures¹². The problem of tertiary structure prediction thus becomes a problem of the arrangement of super-secondary building blocks which have relatively few possible arrangements in a protein.

Received 9 August; accepted 30 November 1982.

1. Chou, P. Y. & Fasman, G. D. *Biochemistry* **13**, 211–245 (1974).
2. Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97–120 (1978).
3. Schulz, G. E. & Schirmer, R. H. *Principles of Protein Structure* (Springer, Berlin, 1979).
4. Palau, J., Argos, P. & Puigdomenech, P. *Int. J. Peptide Protein Res.* **19**, 394–401 (1982).
5. Bussetta, B. & Hospital, M. *Biochim. biophys. Acta* **701**, 111–118 (1982).
6. Bernstein, F. C. *et al.* *J. molec. Biol.* **112**, 535–542 (1977).
7. Cohen, F. E., Sternberg, M. J. E. & Taylor, W. R. *J. molec. Biol.* **156**, 821–862 (1982).
8. Matthews, B. W. *Biochim. biophys. Acta* **405**, 442–451 (1980).
9. Brahms, S. & Brahms, J. *J. molec. Biol.* **138**, 149–178 (1980).
10. Williams, R. W. & Dunker, A. K. *J. molec. Biol.* **152**, 783–831 (1981).
11. Rao, S. T. & Rossmann, M. G. *J. molec. Biol.* **76**, 241–256 (1973).
12. Richardson, J. S. *Nature* **268**, 495–500 (1977).

Along the road to Asilomar

Bernard D. Davis

THE CONJECTURAL dangers from recombinant DNA (rDNA) have failed to materialize, and the public's recent fear of this research has been replaced by a deep interest in its achievements and its promise. It is therefore time for a scholarly analysis of this remarkable affair, with its unprecedented degree of public involvement in a highly technical set of issues.

Sheldon Krimsky, a social scientist at Tufts University, has undertaken the task of providing such an account, based not only on the published record but also on private discussions among scientists (recorded by others in oral histories). The book is scholarly in its chronological presentation and is heavily enough referenced to be a useful source. Unfortunately, however, it is dominated by the author's populist social perspectives: science is too dangerous to be left to the elitist scientists, and only direct public participation can protect the public interest. There is no sign of any reflection on the possibility that excessive public participation might have slowed the eventual resolution of the problem, or that the public's right to know should be balanced with a right to be spared from scaremongering.

The book is thus a curious mixture. The author tries hard to present the arguments on all sides. But although he avoids the strident tone of many earlier critics of rDNA research, he shares their suspicion of the scientific community. The resulting position is paradoxical. The present relaxation of the guidelines is not necessarily wrong, says Krimsky; but in describing each step on the way he offers an unsophisticated analysis that rejects the scientific evidence and judgements supporting the change, while treating with great respect even the most far-fetched contrary arguments. Hence this book is far from the judicious retrospective analysis that is needed. Let me suggest some of the points that such an analysis might consider.

First, one might ask whether or not the extensive public discussion of the hazards was a good

Genetic Alchemy: The Social History of the Recombinant DNA Controversy. By Sheldon Krimsky. Pp.445. ISBN 0-262-11083-0. (MIT Press: 1982.) \$24.95, £19.95.

thing. Many people would say yes. Scientists earned good marks for opening their doors to the public; all sides had their say, in the democratic tradition; and in the end reason prevailed. Nevertheless, I would agree with those who come out with a much less favourable balance-sheet. Large sums of money and much time were diverted from productive research; the United States Congress came close to enacting severely restrictive legislation, which would have been hard to reverse; the inroads into the traditional autonomy of science, and the imposition of an onerous bureaucracy (still present), set a dangerous precedent; the anxiety aroused was an unnecessary burden for the public; and the view of science as a threat was reinforced.

The crux of the matter is that there cannot be conclusive answers to questions about conjectural hazards. In the face of such uncertainty wide publicity is an invitation to emotional reactions at best, and to demagoguery at worst. I would therefore criticize the format of the Asilomar conference, which was convened in 1975 to consider the hazards of rDNA research. A committee of 150, in the glare of world-wide publicity, is not an ideal instrument for evaluating technical issues. This conference, as described by some of those present, rather resembled a religious

revival meeting. The outcome was a finely graded classification of risks, established as though it were based on sound science when in fact it was based on guesswork.

I have long wondered how such a distinguished group of scientists could have accepted such a metaphysical construction, culminating in a fear even of random human DNA as a dangerous material. (Krimsky fails to note that Joshua Lederberg and Jim Watson pleaded in vain against this course.) Krimsky's account provides a possible key. As he relates, the conference was built around the reports of working groups with research backgrounds in different classes of DNA. The members of the Animal Virus Group (chaired by Aaron Shatkin), accustomed to the risks of working with viruses, were the least worried. They handed in (with one dissent) a one-page report, recommending that research with viral recombinants in bacteria should follow already existing guidelines for work on the viruses themselves. In contrast, the report of the Plasmid Working Group (chaired by Richard Novick) filled 35 single-spaced pages. After expressing broad concern over environmental hazards and philosophical matters, it classified experiments into six levels of biohazard and corresponding levels of containment.

It is not clear how much the subsequent adoption of a detailed classification of risk, in the NIH guidelines, depended on this report. The Ashby Committee in Britain came out with a similar classification, but

by a different mechanism. But whatever its ontology, the classification was in effect a certification of undemonstrated risks. It thus provided a foundation on which a handful of dissident scientists could arouse great public anxiety. What should have been a set of judgements about probabilities then degenerated into arguments about an inappropriate, potentially paralytic question: "Can you prove that the following could not happen?"

We must ask why the molecular biologists were willing to air publicly apprehensions that rested so heavily on guesswork and on the extrapola-

IMAGE
UNAVAILABLE FOR
COPYRIGHT
REASONS

tion of already uncertain knowledge. One reason was clearly an admirable sense of moral responsibility, coupled with political inexperience: they did not foresee how their conscientious descriptions of remote possibilities would eventually be interpreted as a conviction of imminent dangers. But perhaps the most interesting contribution of Krimsky's book is the recognition of an additional, cultural factor: the recent widespread loss of confidence in the authority of experts. Brought up to see authority in any form as elitist, and extrapolating from the real dangers from nuclear technology to the putative ones from biology, many young molecular biologists were ambivalent about the future social impact of their field. In this atmosphere the pioneers in recombinant DNA research must initially have felt quite virtuous in showing that scientists could now be open and anti-elitist. Moreover, the loss of nerve in the scientific community became widespread. Thus when the National Academy of Sciences decided to try to help it did not set up the traditional blue-ribbon committee. It held a public forum that gave equal time to all sides: the cautiously optimistic mainstream biologists, the handful of scientific Cassandras and the political activists. This forum was not very helpful. Of course, it is not certain that a more traditional approach could have been more influential in such a charged atmosphere —

but, in retrospect, one must wonder.

The molecular biologists also contributed to the problem by a lack of willingness to listen to biologists in other areas of research. Since molecular biologists had created whatever dangers might exist, and since they would be most affected by any restrictions, it is not surprising that it was they who assumed responsibility for assessing the risks, primarily by experimental tests. And, indeed, the favourable results of several risk experiments did contribute much support for the later relaxation of the guidelines. But from the start experts in other fields, closer to the problem of risk assessment, could have invoked principles that justified more reassuring judgements. Thus investigators of infectious disease deal constantly with pathogenic bacteria that are well adapted to survival in nature, and some were very doubtful that 0.1 per cent foreign DNA in *E. coli* could create an even greater hazard. In addition, as Krimsky concedes (but without being convinced), two Darwinian arguments provided a theoretical framework for rejecting the early scary scenarios: introduction of foreign DNA will inevitably impair the genomic balance that is essential for survival and spread in nature; and since bacteria can take up DNA in nature the recombinants being made in the laboratory could not be a radically novel class after all. Unfortunately, it took time for all these principles to emerge and to be taken seriously, and meanwhile the course of the drama was already set at Asilomar.

Among the lessons that might be drawn I would suggest the following. First, the evaluation of risks must precede decisions about their acceptability; and while the public, through its representatives, should be heavily involved in the latter process, its involvement in the process of technical analysis is likely to be a hindrance rather than a help. Second, it takes time for scientists to see the implications of highly novel developments, and meanwhile the public does not benefit from exposure to transient, frightening hypotheses. Third, mass meetings of scientists are a much poorer mechanism for evaluating controversial issues than the traditional small committee. Fourth, nuclear technology, with its great economic and military pressure to underestimate the real dangers, is a poor model for assessing potential hazards in basic biological research. Finally, the search for absolute security is a will-o'-the-wisp, diverting attention from real hazards and delaying real benefits. With highly conjectural hazards from scientific advance we can do no better than be guided by subjective probabilities, coupled with a sharp watch for early warning signs of tangible risk. □

Bernard D. Davis is Adele Lehman Professor of Bacterial Physiology at Harvard Medical School.

Gas with a slightly pungent taste

J.E. Lovelock

Carbon Dioxide Review: 1982. Edited by William C. Clark. Pp.467. ISBN 0-19-855368-4. (Oxford University Press: 1982.) £22.50, \$35.

THE PAST two decades have seen the publication of many special reports and reviews of environmental problems. Most were out of date at the time of their writing and quite fossilized when published, usually several years later. My astonishment at receiving the *Carbon Dioxide Review: 1982*, printed in the year of its preparation, turned to pleasure as I read its wise words and balanced conclusions, and glanced through its elegantly organized data lists, bibliography and illustrations. It is without doubt the most valued book I have received on our planetary environment. No one interested in or professionally concerned with this topic can afford to be without at least one copy. Any others are sure to be borrowed and never returned.

It takes some audacity to write about the future course of energy use, atmospheric composition and climate. The largest and fastest computer models can only see a few days ahead in weather forecasting. To guess at the climate of the next century seems foolhardy. Yet our present actions, which include the burning of fossil fuel, the clearing of the forests and perhaps the extension of agribusiness to the continental shelves, all have the potential adversely to affect our future. In our anxiety it is all too easy to do nothing but worry or to follow the simplistic way of the puritan environmentalist, find a scapegoat and destroy it now. But what scapegoat and what action? Should we, like the French, choose a nuclear-powered future? Or should we continue with conventional fuels but endeavour to curb their inefficient and wasteful use?

Reading through the *Carbon Dioxide Review* is very easy, so free is it of errors and so well illustrated and edited. Rather than make recommendations for action, the review persuasively puts the case that we need to know a great deal more about the world and about the future developments of human societies. But perhaps its most important message is that there is time to gather this information and that it is more important to do this than to opt in ignorance for some unwise or precipitate action.

My father-in-law sprinkled pepper on his strawberries to bring out the flavour, likewise do reviewers. Pepper is not included among the condiments for this book; instead there are novel and fascinating flavours. Thus C.F. Baes, writing about the role of the oceans, gives for the first time an active role to the biota

RESEARCH IN BRITISH UNIVERSITIES POLYTECHNICS AND COLLEGES

RBUPC

1982

YOUR GUIDE TO
CURRENT RESEARCH IN THE UK

Vol. 1 Physical Sciences £40
ISBN 07123 2003 2

Vol. 2 Biological Sciences £40
ISBN 07123 2004 0

Vol. 3 Social Sciences £30
ISBN 07123 2005 9

Set of 3 volumes (1, 2 & 3) £99
ISBN 07123 2002 4



Available from:

Publications Section (A9),
British Library Lending Division,
Boston Spa, Wetherby,
West Yorkshire, LS23 7BQ

Circle No.14 on Reader Service Card.

and shows how greatly they could affect the flux of CO₂. Then P.R. Bell writes about the massive stores of methane hydrate buried in the permafrost and in the ocean sediments. If this methane were released as a result of global warming by CO₂, then there would be a new and powerful positive feedback leading to further heating.

Our first intimation of the limits to our planetary environment came in the late 1950s, marked by the publication of Rachel Carson's *Silent Spring*. In those times growth was exponential and it was easy to predict doom, even by the end of the century. Now, in the early 1980s, growth is slow and linear; we may even be in a steady state. The geological world has changed also. In the stratosphere there is now a substantial excess of sulphuric acid aerosol and of hydrochloric acid gas, put there by the maverick volcano, El Chichon. This

might bring freezing winters and wretched summers and by all accounts should modify the ozone layer. We may be glad of the tropospheric warming action of the CO₂ blanket and also of the stratospheric cooling by CO₂ to offset the destruction of ozone by the HCl. It is a measure of the stability of the *Carbon Dioxide Review* that the implications of the activity of El Chichon, which came too recently to be considered in the book, barely detract from the value of its information and conclusions.

There is an extensive and ecumenical bibliography, and data lists which look very solid and complete. We have needed a book like this for a long time. My grateful thanks go to William C. Clark and his colleagues for producing it. □

J.E. Lovelock is an independent scientist.

Chemistry: getting the right reaction

H.M. Frey

Reactivity in Organic Chemistry. By Gerhard W. Klumpp. Pp.502. ISBN 0-471-06285-5. (Wiley:1982.) £41.55, \$66.45.

THIS is a translation, and a good one, of *Reaktivität in der Organischen Chemie*, Volumes I, *Produkte, Geschwindigkeiten*, and II, *Übergangszustände*, which were published in 1977 and 1978. However, the opportunity has been taken to add some new material including work published after the original volumes (up to and including 1980). The author's central concern is reactivity of organic compounds and his examples generally relate to reactions that are or have been important from a synthetic point of view.

The first short chapter, entitled "Multiple Reactivity of Organic Compounds", sets the stage for the remainder of the book. Here Klumpp notes that as well as the problems which result from a molecule containing different reacting groups, others occur because the same group (e.g. carbonyl) may react in many ways. He then turns to highlight the area of product orientation, the topics of stereoselectivity and regioselectivity being discussed in relation to a range of system types.

In the following four chapters the topics already introduced are considered first

from the standpoint of thermodynamic and kinetic control, and with regard to their effect on product ratios. Rates or reactions are then thoroughly treated, the various empirical correlation equations (much loved by physical organic chemists) being presented in a comprehensive and detailed way. Transition state theory is also introduced, so that it can be used to discuss relative reactivities including kinetic isotope effects and linear free energy relationships.

An account of the properties of activated complexes forms the concluding chapter. In many ways I found this the most exciting part of the book, ranging as it does from energy surfaces, position of the transition state on the reaction coordinate to orbital symmetry correlations. Here there are some instructive and unusual diagrams, for example a "cinematographic" representation of ¹CH₂ approaching ethylene to form cyclopropane, and an extremely improbable energy surface involving an activated complex of high symmetry together with a simple explanation, when one looks up the reference, of why it is unrealistic.

The book is really packed full of diagrams, tables and examples, and is very up to date. Quantitative data are given wherever possible. This is not a textbook nor is it a monograph, for it assumes too much to be one and is more far-ranging than the other. Rather, it is a book to be read after one has mastered a conventional textbook of organic chemistry. Able third-year honours students and research workers in organic or physical organic chemistry should find it most worthwhile to do so. □

H.M. Frey is Professor of Physical Chemistry at the University of Reading.

A fluid mosaic

N. Michael Green

Lipid-Protein Interactions, Vols 1 and 2. Edited by Patricia C. Jost and O. Hayes Griffith. Vol. 1 pp.338, ISBN 0-471-06457-2; Vol. 2 pp.307, ISBN 0-471-06456-4. (Wiley: 1982.) Vol.1 £62.35, \$99.75; Vol.2 £58.20, \$93.10.

INTERACTIONS between proteins and lipids differ from those in most other protein-ligand systems in that both lipid and protein may be highly aggregated in aqueous media in the absence of a detergent. In consequence, equilibria are difficult to formulate and the subject has lacked a straightforward conceptual and experimental framework.

This is well exemplified by the first of these volumes, in which each non-membranous system is considered in a separate chapter. Although these proteins have been fairly well characterized, the experimental approaches have been so diverse that the systems have only slight relevance for each other. For example, in the first chapter B.W. Matthews gives a lucid account of the unusual three-dimensional structure of bacteriochlorophyll, a trimer of 15-stranded beta sheets, each subunit enclosing seven chlorophyll molecules; the unique configurations of the seven phytol chains are emphasized but in the absence of the exact amino acid sequence little can be said about the interactions which determine these configurations. In contrast, in the next chapter the lack of crystallographic information about serum albumin is compensated by an abundance of chemical evidence, including the complete sequence, which is integrated into a convincing model by Brown and Shockley.

Much of the remainder of this volume deals with pancreatic phospholipase and the family of phospholipid exchange proteins. General reviews of their properties are followed by detailed descriptions of interactions with both monomeric and micellar substrates. It is disappointing that in spite of an impressive variety of experimental evidence, including the complete structure of phospholipase A, the full description of a phospholipid binding site remains tantalizingly out of reach.

The second volume considers membrane proteins from different operational points of view. Two penultimate chapters provide theoretical background to the whole field of protein-lipid interactions. The first, by J. Reynolds, leads from equilibrium studies of the interactions of amphiphiles with soluble proteins to a necessarily less rigorous treatment of membrane proteins. Many useful insights emerge from this and my only regret is that the chapter was not longer. The second, by the editors, gives a theoretical treatment of the exchange of

Research in Europe

NEW from Longman is the fifth edition of *European Research Centres*, edited by Trevor I. Williams, a guide to European organizations conducting or promoting research in science, technology, agriculture and medicine. The directory is in two volumes and for each institution includes details of research projects and a listing of senior staff. Price is £155.

lipid molecules between the boundary layer surrounding the protein and the main bilayer phase in terms of specific protein binding sites for phospholipids, but the problem of defining a binding site for a hydrophobic molecule in a hydrophobic phase is not discussed. The properties of the lipid in the boundary layer are also treated extensively in contributions on the use of ESR and NMR methods. These chapters contain much useful information about the molecular properties of specific membrane systems, and the apparent conflicts which arise from the different time scales relevant to the two methods are clearly explained. It appears that we are approaching the stage at which it will be possible to make useful generalizations about the elusive annulus.

A different source of information about the regions of the polypeptide chain which are located in the lipid phase is provided by

hydrophobic photoactivated reagents. The critical review by Khorana and colleagues gives a valuable perspective on this newly developed field. Unfortunately the mobility of the probes within the bilayer is such that the site of labelling depends more on the reactivity of the group than on its location.

Throughout both volumes there is an emphasis on experimental methods and results. There are appendices on assay methods and syntheses of reagents, and one chapter is devoted to phase transitions of a large number of synthetic lipids. *Lipid-Protein Interactions* is full of well-organized information on a fragmented subject and it paves the way for a further volume to integrate the puzzle in a few years time. □

N. Michael Green is a member of the Division of Biochemistry at the National Institute for Medical Research, Mill Hill, London.

New light on Newgrange, County Meath

J.N. Graham Ritchie

Newgrange: Archaeology, Art and Legend. By Michael J. O'Kelly. Pp.240. ISBN 0-500-39015-0. (Thames and Hudson: 1982.) £16. To be published in the USA in August 1983.

NEWGRANGE in County Meath, built around 3200 BC, is one of the great cathedrals of megalithic religion and is counted high among the archaeological and tourist wonders of Ireland. One Sunday in 1967 I found the tomb closed to the public and, with some trepidation, scaled the fence to marvel at the excavations and the richly decorated kerbstones on the perimeter of the mound that covers the cruciform passage-grave (the burial vault). Professor M.J. O'Kelly's work had been under way for six years by that stage and was to continue until 1975. The visitor today sees a different monument, new and exciting, partly restored in the light of the 14 seasons of excavation work that are distilled into this volume.

Excavation reports, like the results of any scientific endeavour, are something of an acquired taste; but O'Kelly, who sadly died only last year, envisaged the book both as an explanation for the interested visitor as well as the definitive account of the work. It includes a discussion of Newgrange in early Irish literature as well as descriptions of the visits of early antiquaries since the first opening of the tomb in recent times in 1699. These important sections, as well as a detailed corpus of the decorated stones, have been provided by Mrs Claire O'Kelly.

Two other reports in the same genre come to mind: Richard Atkinson's pioneering attempt to present Stonehenge

to the general reader, first published in 1956 (*Stonehenge*, Hamish Hamilton and later Penguin Books), and Leslie Alcock's *'By South Cadbury that is Camelot . . .'* (Thames and Hudson, 1972). *Newgrange* is heavier going than either of these books, and many visitors will continue to prefer the portability, layout and price of Mrs O'Kelly's *Illustrated Guide to Newgrange and the Other Boyne Monuments* (published privately in 1978). Fellow archaeologists, however, will applaud O'Kelly's presentation of a complex excavation in such a compact form; several allied research topics have been or will be published separately. At a time when so much of the information gained through excavation remains unpublished, it is both an achievement and an example to present the work of so many years within the compass of a single volume.

The photographs throughout demonstrate the high quality of the excavation, as well as providing insights into the humour and humanity of the excavator. The decorated stones, some of the earliest attempts at all-over patterning in the British Isles with elaborate spirals, lozenges and chevrons, are recorded in detail for the first time; the corpus is a fine piece of dispassionate illustration, marred only by insensitive lettering. Fully documented too is the exciting discovery that the passage-grave was deliberately aligned to allow the rays of the rising midwinter sun to penetrate the central chamber through the opening known as the 'roof-box'.

The finds made at Newgrange, like those from the excavation of many famous ritual sites, are unremarkable. Thus the detailed catalogue of them seems rather out of

place, especially in a volume designed to appeal to the general visitor. One of the most interesting aspects for the professional, in view of recent discussion about animal remains within the deposits of chambered tombs, is the realization that at Newgrange the bones of rabbits and mountain hares were not deliberate additions to the original tomb-deposits but result from animal activities since the tomb was first opened.

We know when the tomb and covering mound were put up because of a series of radiocarbon determinations; such 'dates' have to be calibrated to allow them to be compared to calendar years in order to take account of refinements since the method of dating was first formulated. O'Kelly has chosen to write in radiocarbon years bc, as is the convention for uncalibrated dates bc. Although he makes this clear in the preface, few readers will be able to make the necessary adjustments without recourse to tables. To write as though radiocarbon time existed, as O'Kelly and many others do, creates a barrier to the clear presentation of the past to the general reader. To the prehistorian, however, the publication of this fine volume is a poignant reminder of the loss of a great archaeologist. □

J.N. Graham Ritchie is an Archaeological Investigator with the Royal Commission on the Ancient and Historical Monuments of Scotland.

Developing a subject

C.J. Leaver

The Molecular Biology of Plant Development. Edited by H. Smith and D. Grierson. Pp.617. ISBN 0-632-00727-3. (Blackwell Scientific/California University Press: 1982.) £35, \$69.

PLANT molecular biology is a new discipline which is evolving from earlier studies in plant physiology and biochemistry. After years of benign neglect and chronic under-funding, it has become a fashionable growth subject with strong biotechnological interest. The sudden demand for graduates with a background in plant biology and an ability to clone and sequence DNA has led to a shortage of qualified individuals. There is thus an increasing number of botanists wishing to learn more about the molecular approach and an even larger number of molecular biologists eager to understand plant development. The title of this book will therefore ensure that it sells well to a broad range of students of all ages.

It is, then, unfortunate that the book contains relatively little *bona fide* molecular biology and, in fact, reflects our

level of ignorance of the molecular basis of plant development. The 19 chapters range from encyclopaedic, rather rambling, reviews, to short, personalized and thought-provoking considerations of the unique aspects of plant development. In this latter category are general accounts of possible control points in plant development, as well as specific considerations of individual developmental systems, which should provoke discussion and (of more importance) encourage experimentation. For the mammalian chauvinists who believe that plants are but green animals, those chapters on photomorphogenesis and leaf development give a balanced overview of the crucial role of light in controlling the growth and differentiation of plants.

The deposition of storage reserves during seed maturation and their subsequent mobilization during germination were among the first developmental systems to be studied at the molecular level in higher plants. The steady progress in these fields up until 1980 is well documented and these chapters will serve as a useful introduction to the exciting results which are currently being published.

The book ends with several well-written contributions highlighting those areas which, in the eyes of government, industry and entrepreneurs, justify much of the current financial investment in plant science. These chapters deal with symbiotic nitrogen fixation and the molecular events underlying tumour induction of Ti-plasmids in dicotyledonous plants. Because of the nature of the problems and the obvious potential economic benefit deriving from an understanding of the molecular mechanisms of nitrogen fixation and the development of gene vectors for plant cells, workers in these areas of plant science were among the first to apply recombinant DNA techniques. The rapid progress described in these chapters demonstrates not only the potential agronomic applications, but also the importance of such systems to the study of genes and gene products involved in the control of cellular growth and differentiation.

Like many large, multi-authored books this volume could have been improved by stricter and more constructive editing thus conveying more of the potential and excitement of the many facets of the subject to the aspiring student. As the editors recognize, the three years it has taken to put the book together has meant that the references (64 pages of them) are nearly all pre-1980 and the more molecular chapters are rather dated. Nonetheless, *The Molecular Biology of Plant Development* contains much of interest. Given the shortage of books on the topic, this should justify its purchase by libraries if not by individuals. □

C.J. Leaver is Reader in the Department of Botany at the University of Edinburgh.

Sand and oil in the wheels of diplomacy

Anthony Sampson

The History of the British Petroleum Company. Vol.1. The Developing Years, 1901-1932. By R.W. Ferrier. Pp.801. ISBN 0-521-24647-4. (Cambridge University Press: 1982.) £35, \$64.50.

THE company histories which have been multiplying over the past few years are an acquired taste for the general reader. If they are to faithfully represent the spirit of the company concerned they are bound to include roll-calls of minor characters, pages of statistics and details of technical negotiations. They cannot conceal the fact that, between moments of high drama and political significance, the day-to-day operations of business are routinely boring.

Yet the workings of big corporations, particularly when they venture abroad, are crucial to our understanding of the contemporary world; many companies have taken major roles in diplomatic policy-making and relations between nations, which too often have been played down by conventional historians of diplomacy.

The history of the Anglo-Iranian Oil Company, now known as British Petroleum or simply BP, is an outstanding example: for most of this century this one company was a key factor in Western relations with the Middle East. This long-awaited volume, produced by the Company's historian and his staff after years of research in the archives, takes the story at a leisurely and meticulous pace, with thickets of footnotes, diagrams of oil distillation techniques and plenty of grey photographs of early oil-rigs and camps. But to the *aficionado* of this art form it is full of treasures, and it provides indispensable new documentation for any serious student of the Middle East.

Dr Ferrier can fill in the background to one of the most dramatic stories of Western impingement on a developing country, together with its succession of sub-plots: first the patient exploration for oil in Iran, by an enterprising financier, William Knox d'Arcy, and an intrepid and stubborn oil-driller, George Reynolds; the negotiations to buy the Company, first by Burmah Oil and then by the British government; next the transformation of both the Company and the country by the world-wide demand for oil; and then the first major confrontations between Company and country, and its ruler, the Shah. These episodes show very clearly the precedents and roots of later disasters, culminating in the two exiles of the later Shah, and the Khomeini revolution against the whole Western connection and way-of-life.

Dr Ferrier does not, like so many company historians, adopt an over-reverent attitude to his subject: he relishes the interplay of awkward personalities, and does not hesitate to apportion blame for some of the more flagrant mistakes. He

gives full play to the recurring rows between the administrators and technicians which feature in so much of British industrial history; and he provides, for instance, a wonderful vignette of the relationship between George Reynolds, running his team of Iranian workers, and the arrogant administrator John Black who was put over him: "he asserted his own status by denigrating the position of others. Imposingly self-righteous, he was both indifferent to and intolerant of the sensibility of others".

The personality of Charles Greenway, or "Champagne Charlie" as he was later depicted, the managing director of the young company, dominates the first part of the story. As Dr Ferrier perceives him, Greenway was "a resilient, formidable and tenacious businessman, decorous, even fastidious, but who could trade punch for punch with his commercial competitors". Greenway was tough and persuasive in his



Selling petrol in the early 1920s. A companion advertisement, aimed at men, began "He wants POWER. . .".

negotiations with Winston Churchill at the Admiralty, and he established a combative tradition of autonomy for the Company which never left it. But he was dangerously uninterested in systems of management, and far too slow in realizing the incompetence of his managing agents, men who saw themselves as gentlemen and who had little technical knowledge.

It was Greenway's successor Sir John Cadman, a coal-mining engineer with experience in government, who was the more far-sighted company statesman. He not only understood how to establish an organization; he also recognized the essential importance of working in partnership with the Iranians themselves. In one of the most revealing episodes in this story, Cadman went to great lengths to try to persuade the Shah, through his minister Timurtash, to enter into a real partnership

with the Company, by taking shares in it — for which he gained the support of the British government. For a short time in 1929, when Cadman went out to the oil fields, there seemed a real chance of achieving such a partnership, which would have been unique for its time.

But Cadman and Timurtash soon discovered they had different concepts of partnership, and Timurtash insisted on nothing less than a permanent quarter-interest in the Company. The negotiations were adjourned in a friendly atmosphere, but soon afterwards the opportunity had gone: the New York stock market collapsed, the British government's relations with Iran deteriorated, and Timurtash was out of favour. It was, as Dr Ferrier says, a tragic lost opportunity, and the Company was never to be so far-sighted again in its dealings with Iran.

This volume ends at the tantalizing point

(at least for those interested in oil history) before the discovery of oil in Kuwait and the transformation of Middle East economics. The author has also annoyingly chosen to postpone the account of the great cartel agreement of 1928, negotiated at Achnacarry Castle, to the next volume. The relationships between BP and its rival companies — particularly Royal-Dutch Shell and Standard Oil of New Jersey — weave in and out of the story, and there is important new information about the proposals to merge BP with Shell. But it was the "As Is" agreement at Achnacarry which brought those relationships to a climax, and it is tiresome to have to wait still longer to read BP's side of that story.

But this volume nonetheless provides valuable new insights into the devious and difficult relationships between oil companies and governments, which played such a part in determining the future of the

Middle East; and into the especially odd relations between BP and the British government which half-owned it. Some readers may be put off by the intricate detail, the confusing spelling of Iranian and Arabic names and the author's mannerisms including a fondness for the double negative. But the detail and the slow pace of the book do have the useful effect of conveying what the oil business must have seemed like at the time — a complex, cautious yet very commercial venture which only occasionally found itself exposed to the diplomatic crises which would critically affect the future of the world. □

Anthony Sampson is author of The Seven Sisters: the Great Oil Companies and the World they Shaped (Hodder & Stoughton, 1975). His most recent book is The Changing Anatomy of Britain, published earlier this year by Hodder & Stoughton.

Low down on the Great Barrier Reef

R.F. McLean

The Geomorphology of the Great Barrier Reef: Quaternary Development of Coral Reefs. By David Hopley. Pp.453. ISBN 0-471-04562-4. (Wiley: 1982.) £49.85, \$79.75.

THERE are many barrier reefs; some are even great, Fiji's 200 km long Great Sea Reef being an example. But there is only one Great Barrier Reef. This reef province is enormous. It has an outer perimeter of 2,300 km and a lagoon up to 260 km wide that incorporates, in an area the size of Britain, some 2,500 separate coral reefs. How does one make sense of such a vast and complex system? David Hopley does it by starting from the theoretical foundations of Darwin, Dana, Davis and Daly and the more recent integrated reef models and evaluates these in the local context. He argues that the essence of the explanation of reef morphology is the extent to which it is karst-induced as opposed to growth determined. Rightly he concludes that no single theory will explain all reefs. He also makes sense of the Great Barrier Reef by systematizing its spatial organization and classifying its features.

Coral reefs are biological and geological entities. Hopley's interpretation is unashamedly that of a geomorphologist. Reefs are seen as morphological features consisting of rocks, sediments, structures and surfaces that result from an array of geological, biological, physical and chemical processes. These processes, past and present, are covered in four of the book's thirteen chapters that are less likely to satisfy the respective specialist readers than a more general audience. Later, Hopley comes into his own when discussing the Reef's evolution in terms of antecedent platforms, sea level changes and reef responses, which ultimately leads to an idealized evolutionary model of the reef over the last 125,000 years. This is a highpoint.

Ten years ago, in 1973, two events of significance for reef study took place on the Great Barrier Reef. One was the Royal Society-Universities of Queensland Expedition led by David Stoddart, the other the Second International Symposium on Coral Reefs held on board ship. Both stimulated a great deal of new research on the Reef by Australian workers as well as an

interest in it by scientists from other countries. What then has been learnt in the past decade? The answer is: a great deal, especially in the geosciences. Some indication of this is given in the four chapters dealing with modern reefal environments and the spatial variation within and between reefs. There is a wealth of recent and hitherto unpublished data in these chapters, much stemming from the author's own researches. Also impressive is the way in which the scale problem is handled, local, regional and whole reef. Expanding the scale even further, the book concludes with a brief examination of the variation on a world scale of the most important influences on coral reef development and a comparison of the Great Barrier Reef with other reef systems.

Nineteen eighty-two was an important year for the coral reef community with the launching of a new journal *Coral Reefs* and the Smithsonian publication of a detailed study of part of the Atlantic barrier system. David Hopley's book certainly tops up the vintage. Much is now known about fringing reefs and barrier reefs. Now equal attention needs to be given to the third of Darwin's reef triplet — atolls. □

R.F. McLean is a Lecturer in the Geography Department of the University of Auckland.

IMAGE
UNAVAILABLE FOR
COPYRIGHT
REASONS

Island in the park — Lizard Island, lying 17 miles off the Queensland coast and 10 miles from the outer reef, in the Great Barrier Reef Marine Park.

Biological Sciences

- MALCOLM, D.C., EVANS, J. and EDWARDS, P.N. (eds). *Broadleaves in Britain: Future Management and Research. Proceedings of a Symposium held July 1982, Loughborough.* Pp.253. ISBN 0-907284-02-7. (Institute of Chartered Foresters: 1982.) £8.
- MANIATIS, T., FRITSCH, E.F. and SAMBROOK, J. *Molecular Cloning: A Laboratory Manual.* Pp.545. Pbk ISBN 0-87969-136-0. (CSH: 1982.) \$40(USA), \$48 (elsewhere).
- MARSHALL, T.C. *Tropical Fishes of the Great Barrier Reef.* Pp.126 + Plates. Pbk ISBN 0-207-14157-6. (Angus & Robertson, London: 1982.) £6.95.
- McLAREN, J.S. *Chemical Manipulation of Crop Growth and Development.* Pp.564. ISBN 0-408-10767-7. (Butterworth: 1982.) £37.50.
- MILKMAN, R. (ed.). *Perspectives on Evolution.* Pp.241. Hbk ISBN 0-87893-528-2; pbk ISBN 0-87893-529-0. (Sinauer Associates: 1982.) Hbk £18.50; pbk £9.80.
- MISLIN, H. and BACHOFEN, R. (eds). *New Trends in Research and Utilization of Solar Energy through Biological Systems. Experientia Supplementum, Vol.43.* Pp.156. ISBN 3-7643-1335-8. (Birkhäuser-Verlag: 1982.) SwFr. 44.
- MOORE, D.M. *Flora Europaea Check-List and Chromosome Index.* Pp.423. ISBN 0-521-23759-9. (Cambridge University Press: 1982.) £30.
- MORRISON, A.R. and STRICK, P.L. (eds). *Changing Concepts of the Nervous System. Proceedings of the 1st Institute of Neurological Sciences Symposium in Neurology held October 1980, Philadelphia.* Pp.826. ISBN 0-12-507750-5. (Academic: 1982.) \$59.
- MOSS, D.W. *Isoenzymes.* Pp.204. ISBN 0-412-22200-0. (Chapman & Hall: 1982.) £15.
- MUIRHEAD-THOMSON, E.C. *Behaviour Patterns of Blood-Sucking Flies.* Pp.224. ISBN 0-08-025497-7. (Pergamon: 1982.) £25, \$50.
- MULDER, G.J., CALDWELL, J. *et al.* (eds). *Sulfate Metabolism and Sulfate Conjugation. Proceedings of an International Workshop held September 1981, Netherlands.* Pp.311. ISBN 0-85066-233-8. (Taylor & Francis: 1982.) £19.
- MUMFORD, R.E. and WHITAKER, J.O. Jr. *Mammals of Indiana.* Pp.537. ISBN 0-253-30387-7. (Indiana University Press: 1982.) £27 (UK), \$45 (North America), \$56.25 (elsewhere).
- NANCOLLAS, G.H. (ed.). *Biological Mineralization and Demineralization. Dahlem Workshop Reports. Life Sciences Research Report 23.* Pp.415. ISBN 3-540-11521-8/0-387-11521-8. (Springer-Verlag: 1982.) Np.
- NATHANSON, J.A. and KEBABIAN, J.W. (eds). *Cyclic Nucleotides. Part I: Biochemistry. Handbook of Experimental Pharmacology, Vol.58, Part 1.* Pp.557. ISBN 3-540-10786-X/0-387-10786-X. (Springer-Verlag: 1982.) DM 390, \$173.20.
- NEURATH, H. and HILL, R.L. (eds). *The Proteins, 3rd Edn., Vol.5.* Pp.704. ISBN 0-12-516305-3. (Academic: 1982.) \$78.50.
- NEWTON, R.F. and ROBERTS, S.M. (eds). *Prostaglandins and Thromboxanes.* Pp.143. ISBN 0-408-10773-1. (Butterworths: 1982.) £15.
- NISONOFF, A. *Introduction to Molecular Immunology.* Pp.204. Pbk ISBN 0-87893-594-0. (Sinauer Associates: 1982.) £8.50.
- NITECKI, M.H. (ed.). *Biochemical Aspects of Evolutionary Biology.* Pp.324. ISBN 0-226-58684-7. (University of Chicago Press: 1982.) \$17.
- NORBERT FREINKEL, M.D. (ed.). *Contemporary Metabolism, Vol.2.* Pp.540. ISBN 0-306-40954-2. (Plenum: 1982.) Np.
- O'CONNOR, W.J. *Normal Renal Function: The Excretion of Water, Urea and Electrolytes Derived from Food and Drink.* Pp.433. ISBN 0-7099-1917-4. (Croom Helm: 1982.) £15.95.
- OLSON, L.C. (ed.). *Virus Infections: Modern Concepts and Status. Microbiology Series, Vol.6.* Pp.304. ISBN 0-8247-1859-3. (Marcel Dekker: 1982.) SwFr. 118.
- OPEKE, L.K. *Tropical Tree Crops.* Pp.312. Hbk ISBN 0-471-10060-9; pbk ISBN 0-471-10066-8. (Wiley: 1982.) Np.
- PACKER, L. (ed.). *Biomembranes Part H: Visual Pigments and Purple Membranes, I. Methods in Enzymology, Vol.81.* Pp.902. ISBN 0-12-181981-7. (Academic: 1982.) \$79.
- PADILLA, G.M. and McCARTY, K.S. Sr. (eds). *Genetic Expression in the Cell Cycle.* Pp.454. ISBN 0-12-543720-X. (Academic: 1982.) \$54.
- PARKINS, E.J. *Brain/Mind: A Description in Terms of the Representation and Processing of Information and Homeostatic Control.* Pp.97. (Haydn House, London: 1982.) £1, \$3.
- PARSON, J.A. (ed.). *Endocrinology of Calcium Metabolism.* Pp.566. ISBN 0-89004-344-2. (Raven: 1982.) \$93.
- PLAA, G.L. and HEWITT, W.R. (eds). *Toxicology of the Liver.* Pp.350. ISBN 0-89004-584-4. (Raven: 1982.) \$55.76.
- POLAK, J.M., BLOOM, S.R. *et al.* (eds). *Basic Science in Gastroenterology — Structure of the Gut.* Pp.488. Hbk ISBN 0-9500593-2-3; pbk ISBN 0-9500593-3-1. (Glaxo: 1982.) Hbk np; pbk £7 (UK), £11 (elsewhere).
- RASHKIND, W.J. (ed.). *Congenital Heart Disease. Benchmark Papers in Human Physiology, Vol.16.* Pp.390. ISBN 0-87933-414-2. (Hutchinson Ross: 1982.) \$55.
- RATLEDGE, C. and STANFORD, J. (eds). *The Biology of the Mycobacteria, Vol.1. Physiology, Identification and Classification.* Pp.522. ISBN 0-12-582301-0. (Academic: 1982.) £41.20, \$84.50.
- REFF, M.E. and SCHNEIDER, E.L. *Biological Markers of Aging. Proceedings of a Conference held June 1981.* Pp.252. (US Department of Health and Human Services: 1982.) Np.
- REISFELD, R.A. and FERRONE, S. (eds). *Melanoma Antigens and Antibodies.* Pp.445. ISBN 0-306-40852-X. (Plenum: 1982.) \$49.50.
- REITER, R.J. (ed.). *The Pincal and Its Hormones. Proceedings of an International Symposium held January 1982. Progress in Clinical and Biological Research, Vol.92.* Pp.295. ISBN 0-8451-0092-0. (Alan R. Liss: 1982.) £20, DM 90.

- RICH, D.H. and GROSS, E. (eds). *Peptides: Synthesis — Structure — Function. Proceedings of the 7th American Peptide Symposium.* Pp.853. ISBN 0-935940-01-4. (Pierce Chemical Company: 1981.) Np.
- RICH, M.A. and FURMANSKI, P. (eds). *Biological Carcinogenesis.* Pp.328. ISBN 0-8247-1635-3. (Marcel Dekker: 1982.) SwFr. 148.
- RINEY, T. *Study and Management of Large Mammals.* Pp.552. ISBN 0-471-10062-5. (Wiley: 1982.) £26.50, \$59.95.
- ROSE, A.H. and MORRIS, J.G. (eds). *Advances in Microbial Physiology, Vol.23.* Pp.268. ISBN 0-12-027723-9. (Academic: 1982.) \$43.50.
- ROŽKOŠŇ, R. A *Biosystematic Study of the European Stratiomyidae (Diptera): Vol.1, Introduction, Beridinae, Sarginae and Stratiomyinae.* Pp.401. ISBN 90-6193-132-0. (Dr W. Junk: 1982.) Dfl.185, \$79.50.
- RUSSELL, A.D. *The Destruction of Bacterial Spores.* Pp.400. ISBN 0-12-604060-5. (Academic: 1982.) £19.20, \$59.50.
- SANDEMAN, D.C. and ATWOOD, H.L. (eds). *Neural Integration and Behavior, Vol.4. The Biology of Crustacea.* Pp.327. ISBN 0-12-106404-2. (Academic: 1982.) \$36.
- SCHREVEI, J., GROS, D. and MONSIGNY, M. *Cytochemistry of Cell Cytoconjugates. Progress in Histochemistry and Cytochemistry, Vol.14, No.2.* Pp.269. ISBN 3-437-10698-8/0-89574-156-3. (Gustav Fischer: 1981.) DM 148, DM 132 (subscribers).
- SCOTT, K.J. and CHAKRAVORTY, A.K. (eds). *The Rust Fungi.* Pp.290. ISBN 0-12-633520-6. (Academic: 1982.) £26.80, \$50.
- SILBERHORN, G.M. *Common Plants of the Mid-Atlantic Coast: A Field Guide.* Pp.256. Hbk ISBN 0-8018-2319-6; pbk ISBN 0-8018-2725-6. (Johns Hopkins University Press: 1982.) Hbk £13.25, \$22.75; pbk np.
- SNOW, D. *The Cotingas: Bellbirds, Umbrellabirds and Their Allies.* Pp.203. ISBN 0-19-858511-X/0-565-00833-1. (Oxford University Press/British Museum (Natural History): 1982.) £30.
- SPECTOR, L.B. *Covalent Catalysis by Enzymes.* Pp.276. ISBN 3-540-90616-9/0-387-90616-9. (Springer-Verlag: 1982.) DM 78, \$34.70.
- SPITZER, N.C. (ed.). *Neuronal Development.* Pp.424. ISBN 0-306-40956-9. (Plenum: 1982.) Np.
- STARCK, D. *Vergleichende Anatomie der Wirbeltiere.* P.1,110. ISBN 3-540-11268-5/0-307-11268-5. (Springer-Verlag: 1982.) DM 480, \$213.20.
- SWILLENS, S. and DUMONT, J.E. (eds). *Cell Regulation by Intracellular Signals. NATO Advanced Study Series, Vol.44.* Pp.334. ISBN 0-306-40980-1. (Plenum: 1982.) Np.
- SZMUNESS, W. *et al.* *Viral Hepatitis. Proceedings of the 1981 International Symposium.* Pp.843. ISBN 0-89168-040-3. (Franklin Institute Press: 1982.) \$69.50.
- TAMM, Ch. (ed.). *Stereochemistry. New Comprehensive Biochemistry, Vol.3.* Pp.342. ISBN 0-444-80389-0. (Elsevier Biomedical/North Holland: 1982.) \$65, Dfl. 140.
- TODD NEWBERRY, A. *Life in the Sea. Readings from Scientific American.* Pp.248. Hbk ISBN 0-7167-1398-5; pbk 0-7167-1399-3. (W.H. Freeman: 1982.) Hbk £18.80; pbk £8.40.
- TOMLINSON, P.B. *Anatomy of the Monocotyledons, VII. Helobiae (Alismatidae).* Pp.559. ISBN 0-19-854502-9. (Oxford University Press: 1982.) £50.
- TRIBE, M. and WHITTAKER, P. *Chloroplasts and Mitochondria, 2nd Edn. The Institute of Biology's Studies in Biology, 31.* Pp.84. Pbk ISBN 0-7131-2828-3. (Edward Arnold: 1982.) £2.75.
- TSUCHIYA, M., ASANO, M. and ODA, M. (eds). *Basic Aspects of Microcirculation. Proceedings of the Tokyo International Symposium held July 1981. International Congress Series 578.* Pp.376. ISBN 0-444-90256-2. (Elsevier/North Holland Biomedical: 1982.) \$93, Dfl. 200.
- UNSWORTH, M.H. and ORMROD, D.P. *Effects of Gaseous Air Pollution in Agriculture and Horticulture.* Pp.532. ISBN 0-408-10705-7. (Butterworth: 1982.) £39.
- USDIN, E. *et al.* *Biochemistry of S-Adenosylmethionine and Related Compounds. Proceedings of a Conference held October 1981, Missouri.* Pp.760. ISBN 0-333-33059-5. (Macmillan Press, London: 1982.) £45.
- VAN DER PIJL, L. *Principles of Dispersal in Higher Plants, 3rd Edn.* Pp.215. ISBN 3-540-11280-4/0-387-11280-4. (Springer-Verlag: 1982.) DM 59.89, \$26.60.
- VARMA, R.S. and VARMA, R. (eds). *Glycosaminoglycans and Proteoglycans in Physiological and Pathological Processes of Body Systems.* Pp.520. ISBN 3-8055-3440-X. (Karger: 1982.) SwFr. 496, DM 595, \$297.
- VEERARAGHAVAN, N. *Studies on Leprosy.* Pp.121. (V.H.S. Medical Centre, India: 1982.) Np.
- VERBRUGEN, G. and VEYS, E.M. (eds). *Degenerative Joints: Test Tubes, Tissues, Models, Man. Proceedings of the 1st Conference held October 1980, Belgium.* Pp.242. ISBN 90-219-0521-3/0-444-90250-3. (Excerpta Medica/Elsevier/North Holland: 1982.) \$63.75, Dfl. 150.
- VOGLER, W.R. (ed.). *Cytopheresis and Plasma Exchange: Clinical Indications. Proceedings of Progress in Pheresis. A National Symposium held in Georgia. Progress in Clinical and Biological Research, Vol.88.* Pp.334. ISBN 0-8451-0088-2. (Alan R. Liss: 1982.) £28, DM 95.
- WEILS, K. and WEILS, E.K. (eds). *Basidium and Basidiocarp: Evolution, Cytology, Function, and Development. Springer Series in Microbiology.* Pp.187. ISBN 3-540-90631-2/0-387-90631-2. (Springer-Verlag: 1982.) DM 89, \$39.60.

Applied Biological Sciences

- ALFANO, R.R. (ed.). *Biological Events Probed by Ultrafast Laser Spectroscopy.* Pp.443. ISBN 0-12-049950-9. (Academic: 1982.) \$59.
- BAND, P.R. (ed.). *Early Detection and Localization of Lung Tumors in High Risk Groups. Recent Results in Cancer Research, Vol.82.* Pp.190. ISBN 3-540-11249-9/0-387-11249-9. (Springer-Verlag: 1982.) DM 98, \$43.60.
- BASSUK, E.L., SCHOONOVER, S.C. and GILL, A.D. (eds). *Lifelines: Clinical Perspectives on Suicide.* Pp.235. ISBN 0-306-40971-2. (Plenum: 1982.) \$25.

BURGER, M.M. and WEBER, R. (eds). *Embryonic Development, Part A: Genetic Aspects*. 9th Congress of the International Society held August–September 1981, Switzerland. Progress in Clinical and Biological Research, Vol. 85A. Part B: Cellular Aspects. Progress in Clinical and Biological Research, Vol. 85B. Part A, Pp. 520; Part B, Pp. 698. Part A, ISBN 0-8451-0163-3; Part B, ISBN 0-8451-0164-1; ISBN 0-8451-0085-8 (set). (Alan R. Liss: 1982.) Part A £40, DM 140; Part B £54.60, DM 191.

CONWAY-RUTKOWSKI, B.L. *Neurological and Neurosurgical Nursing*, 8th Edn. Pp. 803. ISBN 0-8016-1035-4. (C.V. Mosby: 1982.) £16.75.

CORRY, J.E.L., ROBERTS, D. and SKINNER, F.A. (eds). *Isolation and Identification Methods for Food Poisoning Organisms*. The Society for Applied Bacteriology Technical Series No. 17. Pp. 392. ISBN 0-12-189950-0. (Academic: 1982.) £30, \$55.50.

COSTA, E. and TRABUCCHI, M. (eds). *Regulatory Peptides From Molecular Biology to Function*. Advances in Biochemical Psychopharmacology, Vol. 33. Pp. 588. ISBN 0-89004-797-9. (Raven: 1982.) \$73.16.

DARGAN, K.S., SINGH, O.P. and GUPTA, I.C. *Crop Production in Salt-Affected Soils*. Pp. 276. (Oxford & IBH, Calcutta: 1982.) Rs. 72.

DE NICOLA, A., BLAQUIER, J. and SOTO, R.J. (eds). *Physiopathology of Hypophysial Disturbances and Diseases of Reproduction*. Progress in Clinical and Biological Research, Vol. 87. Proceedings of the International Symposium held July 1981, Argentina. Pp. 331. ISBN 0-8451-0087-4. (Alan R. Liss: 1982.) £28.90, DM 101.

GARTNER, K. *et al.* (eds). *Research Animals and Concepts of Applicability to Clinical Medicine*. Experimental Biology and Medicine: Monographs on Interdisciplinary Topics, Vol. 7. Pp. 234. ISBN 3-8055-3492-2. (Karger: 1982.) SwFr. 148, DM 177, \$88.75.

LASKIN, A.I. (ed.). *Advances in Applied Microbiology*, Vol. 28. Pp. 282. ISBN 0-12-002628-7. (Academic: 1982.) \$35.

LEVI, S. (ed.). *Ultrasound and Cancer*. Proceedings of the 1st International Symposium held July 1982, Brussels. Invited Papers and Selected Free Communications. Pp. 364. ISBN 90-219-0587-6/0-444-90270-8. (Excerpta Medica/Elsevier Science/North Holland: 1982.) \$80.75, Dfl. 190.

LIEBERMAN, H.A. and LACHMAN, L. (eds). *Pharmaceutical Dosage Forms: Tablets*, Vol. 3. Pp. 472. ISBN 0-8247-1698-1. (Marcel Dekker: 1982.) SwFr. 170.

LIFSHTITZ, F. (ed.). *Carbohydrate Intolerance in Infancy*. Clinical Disorders in Pediatric Nutrition Series, Vol. 1. Pp. 272. ISBN 0-8247-1747-3. (Marcel Dekker: 1982.) SwFr. 105.

MANSFIELD, P. and MORRIS, P.G. *NMR Imaging in Biomedicine Supplement 2: Advances in Magnetic Resonance*. Pp. 354. ISBN 0-12-025562-6. (Academic: 1982.) \$49.50.

MCMICHAEL, A.J. and FABRE, J.W. (eds). *Monoclonal Antibodies in Clinical Medicine*. Pp. 664. ISBN 0-12-485580-6. (Academic: 1982.) £34, \$63.

MOORE, M.A.S. (ed.). *Maturation Factors and Cancer*. Progress in Cancer Research and Therapy, Vol. 23. Pp. 406. ISBN 0-89004-596-8. (Raven: 1982.) \$60.76.

PRAKASH, O. (ed.). *Computers in Critical Care and Pulmonary Medicine*, Vol. 2. Pp. 247. ISBN 0-306-40911-9. (Plenum: 1982.) \$39.50.

RICCARDI, V.M., MULVIHILL, J.J. and WADE, W.M. (eds). *Neurofibromatosis (Von Recklinghausen Disease): Genetics, Cell Biology, and Biochemistry*. Advances in Neurology, Vol. 29. Pp. 282. ISBN 0-89004-497-X. (Raven: 1982.) Np.

ROSE, A.H. (ed.). *Fermented Foods*. Economic Microbiology, Vol. 7. Pp. 330. ISBN 0-12-596557-5. (Academic: 1982.) £29.20, \$60.

SELIKOFF, I.J. and HAMMOND, E.C. (eds). *Brain Tumors in the Chemical Industry*. Annals of the New York Academy of Sciences, Vol. 381. Pp. 364. Hbk ISBN 0-89766-151-6; pbk ISBN 0-89766-152-4. (New York Academy of Sciences: 1982.) Hbk np; pbk \$75.

SETLOW, J.K. and HOLLAENDER, A. (eds). *Genetic Engineering: Principles and Methods*, Vol. 4. Pp. 287. ISBN 0-306-41113-X. (Plenum: 1982.) Np.

SHAW, L.M., ROMAS, N.A. and COHEN, H. (eds). *Prostatic Acid Phosphatase Measurement: Its Role in Detection and Management of Prostatic Cancer*. Annals of the New York Academy of Sciences, Vol. 390. Pp. 147. Hbk ISBN 0-89766-170-2; pbk ISBN 0-89766-171-0. (New York Academy of Sciences: 1982.) Hbk np; pbk \$30.

SPECTOR, S. and BACK, N. (eds). *Modern Methods in Pharmacology*, Vol. 1. Pp. 102. ISBN 0-8451-2500-1. (Alan R. Liss: 1982.) £12.

SPIEGEL, H.E. (ed.). *Clinical Biochemistry*. Contemporary Theories and Techniques, Vol. 2. Pp. 316. ISBN 0-12-657102-3. (Academic: 1982.) \$39.50.

TAKASHIMA, S. and POSTOW, E. (eds). *The Interaction of Acoustical and Electromagnetic Fields with Biological Systems*. A Collection of Papers from the Symposium honoring Professor H.P. Schwan on the occasion of his 65th birthday held November 1980, Philadelphia. Progress in Clinical and Biological Research, Vol. 86. Pp. 196. ISBN 0-8451-0086. (Alan R. Liss: 1982.) £22.60, DM 79.

THOMPSON, D.M.P. (ed.). *Assessment of Immune Status by the Leukocyte Adherence Inhibition Test*. Pp. 380. ISBN 0-12-689750-6. (Academic: 1982.) \$45.

VAHOUNY, G.V. and KRITCHEVSKY, D. (eds). *Dietary Fiber in Health and Disease*. Pp. 330. ISBN 0-306-40926-7. (Plenum: 1982.) \$37.50.

VENULET, J. (ed.). *Assessing Causes of Adverse Drug Reactions with Special Reference to Standardized Methods*. Pp. 233. ISBN 0-12-717350-1. (Academic: 1982.) £15.80, \$29.50.

VON BALLAY, G. and GREGUSS, P. (eds). *Optics in Biomedical Sciences*. Proceedings of the International Conference held September 1981, Austria. Springer Series in Optical Sciences, Vol. 31. Pp. 274. ISBN 3-540-11666-4/0-387-11666-4. (Springer-Verlag: 1982.) DM 84, \$37.30.

WORLD HEALTH ORGANIZATION. *Arsenic*. Environmental Health Criteria 18. Pp. 174. ISBN 92-4-154078-8. (WHO: 1981.) SwFr. 20.

WORLD HEALTH ORGANIZATION. *Manual on Environmental Management for Mosquito Control, With Special Emphasis on Malaria Vectors*. WHO Offset Publication No. 66. Pp. 283. ISBN 92-4-170066-1. (WHO: 1982.) SwFr. 22.

Anthropology

LORD, A.R. (ed.). *A Stratigraphical Index of Calcareous Nannofossils*. Pp. 192. ISBN 0-85312-326-8/0-470-27338-0. (Ellis Horwood/Halsted Press: 1982.) £30, \$60.30.

RUSSELL, D.A. and RICE, G. (eds). *K-Tec II: Cretaceous–Tertiary Extinctions and Possible Terrestrial and Extraterrestrial Causes*. Proceedings of the Workshop held May 1981, Ottawa. Series Syllogeus No. 39. Pp. 151. (National Museum of Natural Sciences: 1982.) Np.

WHALLON R. and BROWN, J.A. (eds). *Essays on Archaeological Typology*. Pp. 200. Hbk ISBN 0-942118-14-6; pbk ISBN 0-942118-15-4. (Center for American Archaeology Press: 1982.) Hbk \$15; pbk \$9.

General

ANGELOGLU, C. and SCHOFIELD, J. (eds). *Successful Nature Photography: How to Take Beautiful Pictures of The Living World*. Pp. 240. ISBN 0-00-411899-5. (Collins: 1982.) £12.95.

ASQUITH, P.D. and HACKING, I. (eds). *PSA 1978. Proceedings of the 1978 Biennial Meeting of the Philosophy of Science Association*, Vol. 2. Pp. 578. ISBN 0-917586-10-7. (Philosophy of Science Association: 1982.) \$22.50.

BALINSKI, M.L. and YOUNG, H.P. *Fair Representation: Meeting the Ideal of One Man, One Vote*. Pp. 191. ISBN 0-300-02724-9. (Yale University Press: 1982.) £21.

BREUER, R. *Contact with the Stars: The Search for Extraterrestrial Life*. Pp. 292. Hbk ISBN 0-7167-1355-1; pbk ISBN 0-7167-1389-6. (W.H. Freeman: 1982.) Hbk £11.95; pbk np.

CROSS, J. and FARRER, D. *Dust Explosions*. Pp. 248. ISBN 0-306-40871-6. (Plenum: 1982.) \$37.50.

GREENFIELD, R.A., RYAN, M.K. and MULVIHILL, J.E. (eds). *Human Subjects Research: A Handbook for Institutional Review Boards*. Pp. 291. ISBN 0-306-40920-8. (Plenum: 1982.) \$25.

HEADY, H.F. and HEADY, E.B. *Range and Wildlife Management in the Tropics*. Pp. 140. ISBN 0-582-60896-1. (Longman: 1982.) £8.82.

HERSHEY, R.L. *How to Think with Numbers*. Pp. 133. Pbk ISBN 0-86576-014-1. (William Kaufmann: 1982.) £5.60.

KANTOROVICH, L.V. and AKILOV, G.P. *Functional Analysis*, 2nd Edn. Pp. 588. Hbk ISBN 0-08-023036-9; pbk ISBN 0-08-026486-7. (Pergamon: 1982.) Hbk £45, \$100; pbk £10, \$25.

KAPLAN, N.O. and ROBINSON, A. (eds). *From Cyclotrons to Cytochromes: Essays in Molecular Biology and Chemistry*. Pp. 887. ISBN 0-12-397580-8. (Academic: 1982.) \$64.

KASCH, F. *Modules and Rings*. Pp. 336. ISBN 0-12-400350-8. (Academic: 1982.) £33.80, \$69.50.

LEWIN, M., ATLAS, S.M. and PEARCE, E.M. (eds). *Flame-retardant Polymeric Materials*, Vol. 3. Pp. 238. ISBN 0-306-40868-6. (Plenum: 1982.) \$35.

LOURIE, J.A. *Medical Eponyms: Who Was Coudé?* Pp. 207. Pbk ISBN 0-272-79643-3. (Pitman: 1982.) £4.95.

MATTHEWS, P.H. *Do Languages Obey General Laws?* Pp. 30. Pbk ISBN 0-521-28963-7. (Cambridge University Press: 1982.) £1.95.

OKOSHI, T. *Optical Fibers*. Pp. 299. ISBN 0-12-525260-9. (Academic: 1982.) \$39.

ROEDDER, E. (ed.). *Fluid Inclusion Research*. Proceedings of COFI, Vol. 11, 1978. Pp. 291. Pbk ISBN 0-472-02011-0. (University of Michigan Press: 1982.) \$10.

RUTTAN, V.W. *Agricultural Research Policy*. Pp. 369. Hbk ISBN 0-8166-1101-7; pbk ISBN 0-8166-1102-5. (University of Minnesota Press: 1982.) Hbk \$32.50; pbk \$13.95.

SHARKANSKY, I. *Public Administration: Agencies, Policies and Politics*. Pp. 393. ISBN 0-7167-1321-7. (W.H. Freeman: 1982.) £16.95.

SIES, H. (ed.). *Metabolic Compartmentation*. Pp. 520. ISBN 0-12-642750-X. (Academic: 1982.) £43.60, \$89.50.

SKINNER, B.J. (ed.). *The Solar System and Its Strange Objects*. Readings from American Scientist. Pp. 193. Pbk ISBN 0-913232-84-X. (William Kaufmann: 1982.) £8.40.

SPOEHR, K.T. and LEHMKUHLE, S.W. *Visual Information Processing*. Pp. 298. Hbk ISBN 0-7167-1373-X; pbk ISBN 0-7167-1374-8. (W.H. Freeman: 1982.) Hbk £16.90; pbk £9.80.

STEIN, S. *The Science Book*. Pp. 285. ISBN 434-96460-3. (Heinemann: 1982.) £4.95.

TERESI, D. (ed.). *Omni's Continuum: Dramatic Phenomena from the New Frontiers of Science*. Pp. 248. Pbk ISBN 0-283-98886-X. (Sidgwick & Jackson: 1982.) Hbk £8.95; pbk £5.95.

THIELHEIM, K.O. (ed.). *Primary Energy: Present Status and Future Perspectives*. Pp. 371. Pbk ISBN 3-540-11307-X/0-387-11307-X. (Springer-Verlag: 1982.) DM 71, \$31.60.

TSAO, G.T. (ed.). *Annual Reports on Fermentation Processes*, Vol. 5. Pp. 323. Pbk ISBN 0-12-040305-6. (Academic: 1982.) \$28.50.

UNESCO. *Manual de Experimentos Químicos, tomo 3: Estequiometría, Termoquímica Equilibrios Químicos*. Pp. 723. (Unesco: 1982.) Np.

WHALLEY, J.I. *Pliny the Elder: Historia Naturalis*. Pp. 47. ISBN 0-283-98905-X. (Sidgwick & Jackson, London: 1982.) £6.95.

WHITTLE, P. *Optimization Over Time: Dynamic Programming and Stochastic Control*, Vol. 1. Pp. 317. ISBN 0-471-10120-6. (Wiley: 1982.) \$42, £19.50.

WORLD HEALTH ORGANIZATION. *Waste Discharge into the Marine Environment: Principles and Guidelines for the Mediterranean Action Plan*. Pp. 422. ISBN 0-08-026194-9. (Pergamon: 1982.) £40, \$80.

WRIGHT, W. *The Social Logic of Health*. Pp. 215. ISBN 0-8135-0948-3. (Rutgers University Press: 1982.) \$14.95.

YAFEE, S.I. *Prohibitive Policy: Implementing the Federal Endangered Species Act*. Pp. 239. ISBN 0-262-24024-6. (MIT Press: 1982.) £12.25.